NOTES ON BLOOD-SERUM THERAPY

W. JOWETT

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PREVENTIVE INOCULATION

AND

TOXIN & SERUM DIAGNOSIS

For Veterinary Practitioners and Students

BY

WALTER JOWETT, F.R.C.V.S., D.V.H.

FORMERLY DEMONSTRATOR OF COMPARATIVE PATHOLOGY AND BACTERIOLOGY IN THE UNIVERSITY OF LIVERPOOL

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PREFACE

In this little volume I have endeavoured to give a short account of the subject of 'Immunity,' and of the Vaccines, Sera, and Toxines used in present-day veterinary practice for the prevention, treatment, and diagnosis of disease.

In compiling these notes the works of many well-known writers have been consulted. These have been duly acknowledged in the text, but I desire especially to express my indebtedness to those of Professors Stewart Stockman, Sir John McFadyean, Hewlett, and Dr. Theiler.

I have to thank Colonel F. Smith for permission to reproduce from his 'Manual of Veterinary Hygiene' Fig. 27, and Professor W. Owen Williams for the loan of a photograph from which the drawing for Fig. 33 was prepared.

W. J.

November, 1906.

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IMMUNITY

Immunity—Antitoxic and antimicrobic sera—Theories of immunity—Metschnikoff's phagocytic theory—Ehrlich's side-chain theory—Opsonic immunity—Opsonins—Hæmolysis—Cytolysis—Agglutinins—Præcipitins.

'When an organism subjected to the action of some influence noxious to certain other organisms is found to be insusceptible to it, that organism is said to be **immune** from that particular noxa' (Hektoen).

The absence or loss of defensive power against such noxious influence characterises the condition known as **susceptibility**.

Example.—Rinderpest. This is essentially a disease of ruminants; horses never contract the malady. In other words, horses are *immune*, whilst ruminants are *susceptible* to rinderpest.

Immunity may be acquired as a result of one attack of certain infective diseases—one attack of rinderpest secures

to the subject after recovery freedom from a second; the same applies to variola and many other diseases.

Exactly in the same way that one attack of such diseases may protect the animal against a second, so artificial inoculation with pathogenic bacteria or their products may also produce immunity.

The term **natural immunity** (or hereditary immunity), as the name implies, indicates that immunity has been enjoyed by the animal from birth, and has not resulted from any reaction occurring in it during its lifetime; if the immunity *has* resulted from changes during its lifetime it is spoken of as **acquired immunity**.

The word 'immunity' really means absolute freedom or exemption, but in medicine its meaning is not so strictly limited. Complete insusceptibility is referred to as **absolute immunity**, whilst for lesser degrees of insusceptibility, the animal still showing a marked resistance to infection, we reserve the term **relative immunity**.

Example.—Glanders. This is essentially a disease of the equine species, from which it is occasionally transmitted to man and certain other animals. Healthy swine do not contract the disease, but on rare occasions it has been conveyed to young and debilitated pigs. Swine, then, are possessed of a relative immunity against glanders.

In the course of an infection the animal organism does not remain inactive, but by bringing into action all its defensive powers it endeavours to repel the invading microparasites: these may be engulfed, destroyed, and removed by the phagocytes; but the animal body possesses still another line of defence—namely, the power of forming **antidotes** to neutralise or annul the poisonous products of bacteria. These antidotal substances appear in the animal juices, and especially in the blood-serum.

The defensive bodies existing normally in the blood serum are know as **alexins**. They are of a proteid nature, and originate from leucocytes, and probably from other animal cells.

Substances which appear only during infection (whether natural or artificial) are known as **antibodies**.

Another different class of substances also result from infection, and appear in the blood-stream; these possess the power of clumping or agglutinating living or dead bacteria, and are known as **agglutinins**. They are closely allied to, but not identical with, **bacteriolysins**, which cause solution and disintegration of bacteria.

The exact nature of these bodies has not been accurately determined; they are probably proteid, and may be of the nature of enzymes or unorganised ferments.

The history of artificial immunity began with the introduction of vaccination by Jenner. Pasteur afterwards introduced a method of protecting animals against fowl cholera. Later it was found that immunity could be produced against many infective diseases by inoculating susceptible animals with attenuated (weakened in virulence) cultures of bacteria or their products—*e.g.*, anthrax, rabies, tetanus, etc.

Finally, it was discovered that the **blood-serum** of animals so immunised could be utilised to prevent and cure disease in other animals.

The blood-serum of an animal possessing these properties is known as an **antiserum**.

There are two varieties of anti-sera.

1. **Antibacterial or Antimicrobic.**—The blood-serum of animals infected with gradually increasing doses of bacteria (living or dead) or cultures, acquires the power of dissolving and disposing of the organisms in question.

Example.—Antistreptococcic serum.

2. **Antitoxic Serum.**—The blood-serum of animals injected with the toxins (products of bacterial growth only—no bacilli) acquires the power of neutralising such toxin, either when mixed with it in the body of another animal or experimentally in a test-tube.

Antitoxic sera against tetanus, diphtheria (man), snake venom, etc., have been made practically useful.

This rapid form of curative inoculation with **antisera** may also be employed as a prophylactic measure, but it yields only **temporary** immunity. It has been termed **passive** immunity by Ehrlich, to distinguish it from the **active** and more lasting immunity yielded by an actual attack of the disease, or by inoculation with bacteria or their toxic products.

Behring applies the term **isopathic** immunity to active, and **antitoxic** to passive immunity.

Active immunity is slowly acquired, but lasts a long time. Passive immunity acts almost immediately, but soon passes away. Acquired immunity may be either active or passive. Passive immunity is always acquired.

Active immunisation, also termed **vaccination** or **protective inoculation**, must produce immunity before the disease is acquired.

Passive immunisation, also termed **serotherapy** or **Antitoxic treatment**, neutralises the toxin already circulating in the blood before it has entered into combination with the tissues.

Theories of the Nature of Immunity.

The present-day theories of immunity may be divided into two classes—

1. **The Cellular Theories**, which assume that the body is protected **directly** by means of its living cells.

2. **The 'Humoral' Theories** assume that the body is protected by means of the extracellular fluids (notably the blood-serum), which either (*a*) destroy the microbes, (*b*) render inert their toxins, or (*c*) render the specifically irritable cells of the body insusceptible to the poison.

Buchner attributes the bactericidal power of the blood to the presence in it of alexins, bodies which by Hankin are known as 'the defensive proteids,' and by Kossel and Vaughan are believed to be derived from nuclein and nucleinic acid.[1]

Both cellular and humoral theorists agree that the bactericidal, antitoxic, and attenuating substances must be derived IN THE FIRST PLACE *from the body cells.*

Firstly, we will consider briefly the **cellular theories**, and of these the chief is—

Metschnikoff's Phagocytosis Theory.

Metschnikoff believes that if the phagocytic properties are possessed in such degree by an animal that a given pathogenic microbe cannot develop in its body, then the animal is naturally immune.

If, during the course of an infection, the microbic products *repel* the phagocytes (**negative chemotaxis**), as in the acute septicæmias, then the parasites increase in the body, as in a tube of culture medium, and soon kill the animal.

If, however, in the course of an infection, the microbic products *attract* the phagocytes (**positive chemotaxis**), then the latter may engulf, destroy, and remove the invaders, the animal recovers and acquires immunity.

But the incorporation of micro-organisms by phagocytes

[1] Nuclein is a proteid substance ; its existence was first demonstrated in the nuclei of cells of the body—hence the name. It is associated with a phosphorus-containing material of acid reaction —nucleinic acid.

does not always or necessarily lead to the death of the former; their products may, on the contrary, kill the phagocyte, so that in some diseases, although there is well-marked phagocytosis, yet the animal succumbs.

Example.—Swine erysipelas (see Figs. 1 and 2).

In other cases the resisting power of the bacteria and phagocytes is more evenly balanced; the chronic course as well as the localised form of these diseases has been explained thereby. In these cases the bacteria may multiply even within the cells.

Example.—Chronic tuberculosis (see Figs. 3 and 4).

As a rule, it is found that the more virulent the bacteria, the less marked is the accumulation of leucocytes and phagocytosis.

Example.—If an **attenuated** culture of anthrax bacilli be injected subcutaneously into the ear of a rabbit, an enormous collection of leucocytes and well-marked phagocytosis results, whereas if a **virulent** culture be injected, an intense **serous** inflammation follows, the fluid containing very few leucocytes.

The virulent culture evidently repels (negative chemotaxis), whilst the weak attracts the leucocytes (positive chemotaxis).

In many instances the first attack of a disease is followed by negative chemotaxis, which in the course of the infection gives way to positive chemotaxis, phagocytosis, and, after recovery, to immunity.

'The activity of the phagocytes is artificially alterable.' An animal ordinarily susceptible to a given infection can, through stimulation of the phagocytes to greater activity, be rendered artificially immune. If the stimulation be only transient in effect, then we have produced only the passive immunity of Ehrlich; if more prolonged or permanent, active immunity.

Metschnikoff has shown that in infection with *Vibrio*

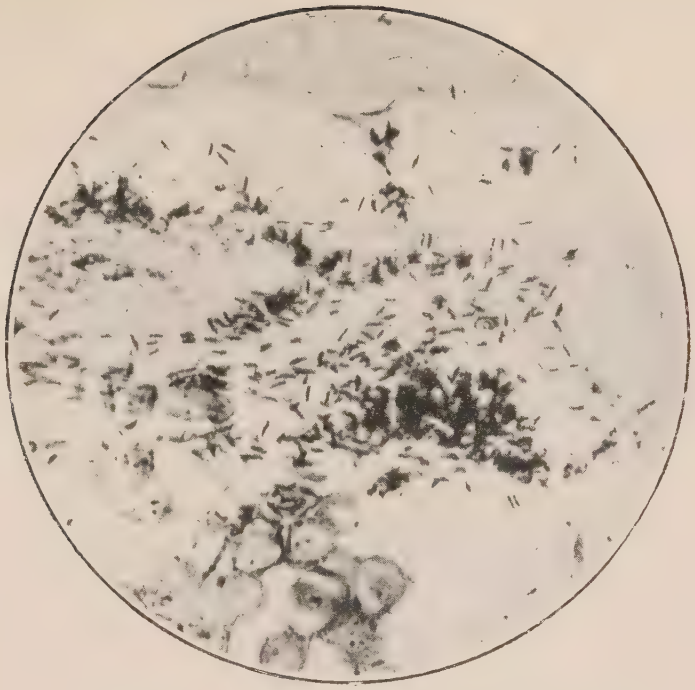


FIG. 1.—SWINE ERYSIPELAS: MICROPHOTOGRAPH OF BLOOD-SMEAR FROM AFFECTED ANIMAL (×900).

Numerous bacilli are shown, some between the corpuscles, many massed in the interior of leucocytes.

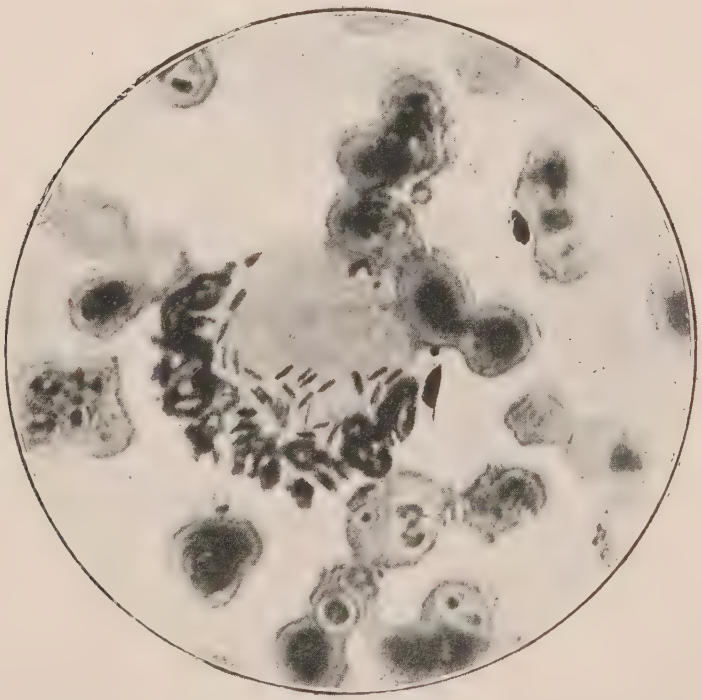


FIG. 2.—SWINE ERYSIPELAS: SAME PREPARATION AS FIG. 1 (×1680). LEUCOCYTE CONTAINING BACILLI.

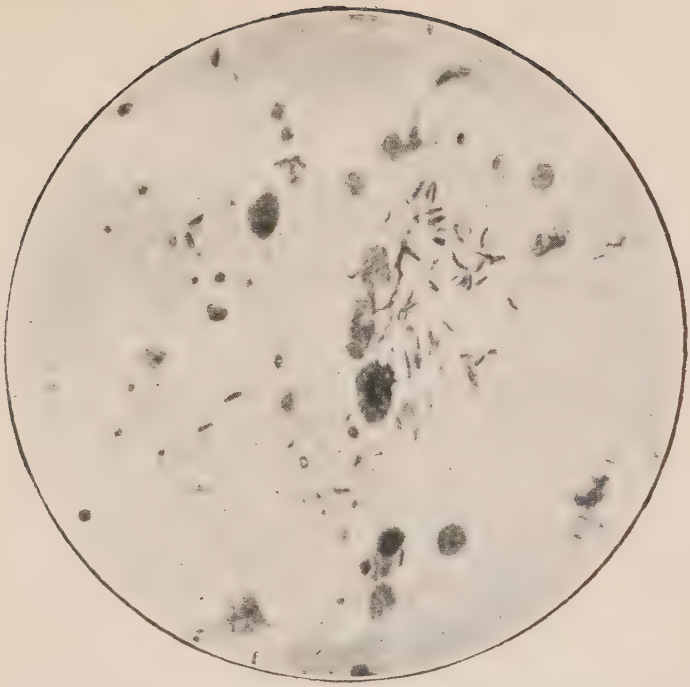


FIG. 3.—TUBERCULOSIS OF MESENTERIC GLAND: HORSE (×800).
In this photograph a giant cell is shown with numerous bacilli in its interior.

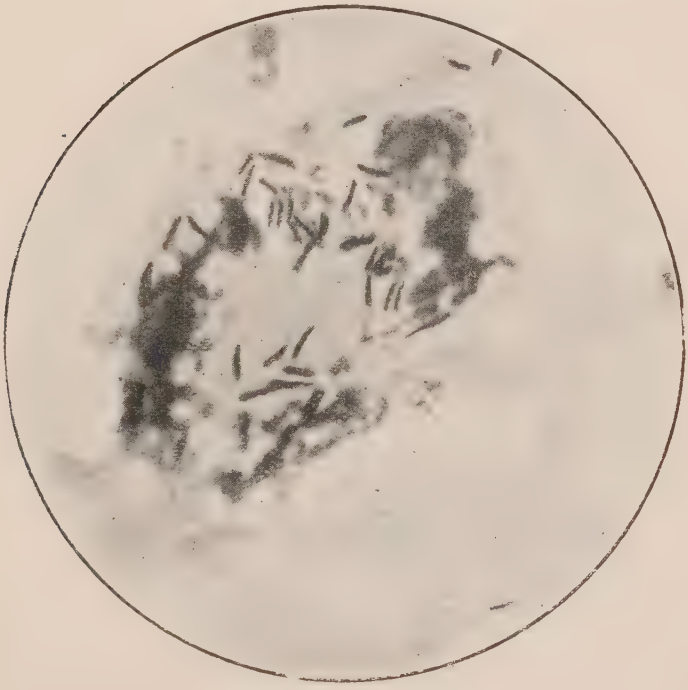


FIG. 4.—TUBERCULOSIS: SAME PREPARATION AS FIG. 3 (×1500).
GIANT CELL CONTAINING BACILLI.

Metschnikovi[1] the phagocytes of unprotected animals do not take up the bacteria, whilst in artificially immunised animals they are loaded with them.

'It appears, therefore, that the phagocytes are active according to the degree of immunity.'

Metschnikoff maintains that the pathogenic bacteria are destroyed by the phagocytes, and immunity depends upon the activity of these latter. Any bactericidal (bacteria-dissolving) action of the body juices, he holds, results from the leucocytes—in fact, he considers that all the lysins in the blood originate from the leucocytes.

'Whereas Buchner believes the alexins (solvent substances) to be true secretory products of the leucocytes, Metschnikoff affirms that they originate in the breaking up of the leucocytes (phagolysis)—*i.e.*, they are decomposition products—and he bases his belief chiefly on the work of his pupil, Gengou, who showed that, although the serum was rich in alexin, the plasma contained none at all' (Wassermann).

Metschnikoff believes, then, that the leucocytes are the *essential* agents in immunity, acting either by incorporating and digesting the micro-organisms, or by giving origin to products which render inert their toxins, and that any dissolving action on bacteria or cells the body juices may possess is due to the presence therein of substances which have escaped from the leucocytes.

Ehrlich explains the way in which immunity is brought about in a somewhat different manner. This scientist has elaborated a theory now generally known as—

[1] The *Vibrio Metschnikovi* was isolated by Gamaleia from the intestines of chickens affected with a disease resembling fowl cholera. He succeeded in immunising pigeons and guinea-pigs (very susceptible to infection with this organism) by inoculating them with cultures of *V. Metschnikovi* killed by heat.

Ehrlich's Lateral Chain Theory.

He assumes that the normal protoplasm of the body cells is built up of highly complex organic molecules, consisting of two groups :

(1) A central stable group, having attached to it—

(2) Lateral, far less stable chains of atoms — 'lateral chains,' 'side chains,' or 'receptors.'

Under ordinary circumstances the function of these lateral chains is to take up molecules of food for the nourishment of the cell ; the process of nutrition consists simply in the joining or coupling of the side chains of food-stuffs to suitable side chains or receptors of the body cells, but the two sets of side chains, those of the animal cells and those of the food-stuffs, must accurately 'fit' each other (Fig. 5), otherwise there can be no union—as, for instance, a male and female screw are useless if not 'fitting' each other.

The side chains of the body cells may, however, unite with substances other than food-stuffs, with substances which may do harm to the cell instead of benefit ; poisons, in fact, do injury if they find lateral chains (receptors) with which they can unite. Now, as lateral chains become united to these poisons the cells are stimulated to form new lateral chains to take their place, and (provided that the animal survives the primary injury) these are produced **in excess** of the original number. The combination of the receptor groups of the living cell with the receptive groups of the poison produces damage to the cellular protoplasmic matter of the former, and repeated damage followed by recovery is attended by overproduction or hypertrophy—hence the resulting **excess** of side chains.

Ehrlich's Theory as applied to Antitoxic Sera.

If we inject into an animal gradually increasing doses of the **toxins** of a pathogenic micro-organism, not only does

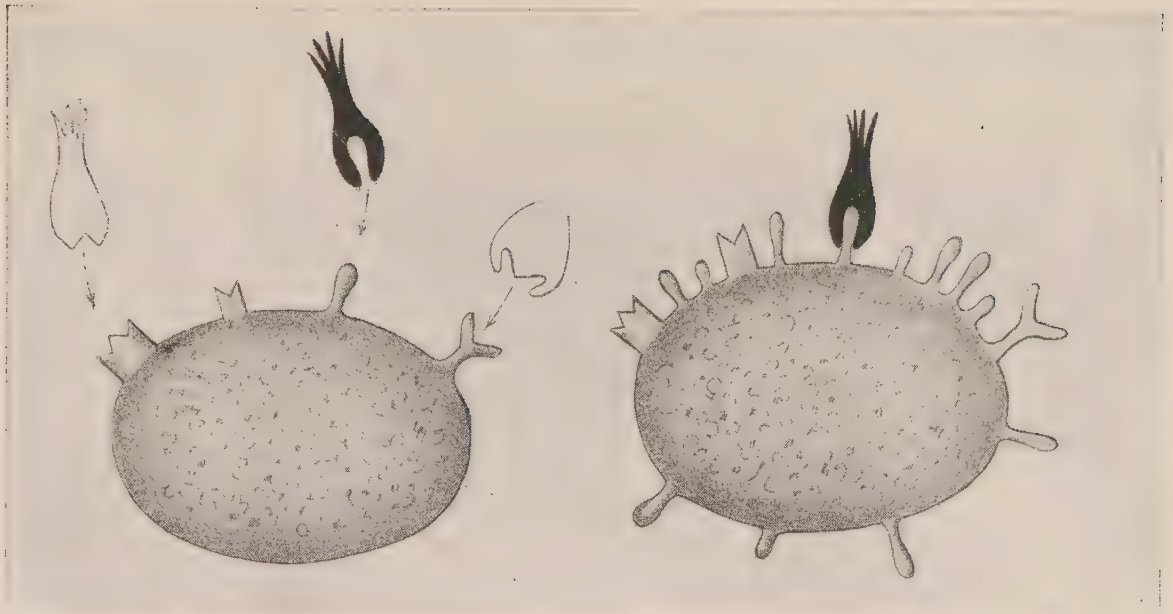


FIG. 5.—DIAGRAM TO REPRESENT THE NORMAL CELL WITH ITS VARIOUS COMBINING GROUPS (SIDE CHAINS).

Each side chain is receptive only for the specific ('fitting') form of haptophore.

FIG. 6.—FIRST STAGE IN THE FORMATION OF ANTITOXIN.

A receptor and haptophore are united, and new receptors have formed to take the place of those combined.

(*After Ehrlich.*)

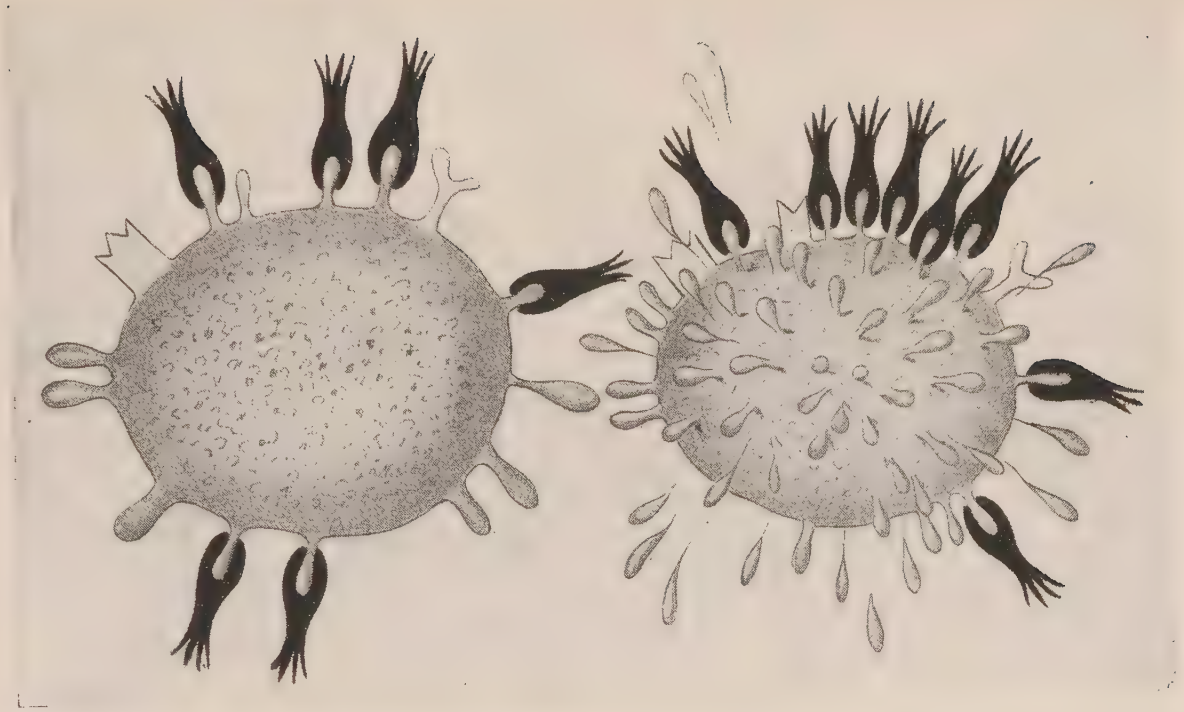


FIG. 7.—SECOND STAGE: A NUMBER OF HAPTOPHORES LINKED ON TO THE NEW-FORMED RECEPTORS.

FIG. 8.—THIRD STAGE: ANTITOXIN BEGINNING TO BE FORMED.

So many receptors have now formed that the cell is encumbered, and therefore liberates some of them.

(*After Ehrlich.*)

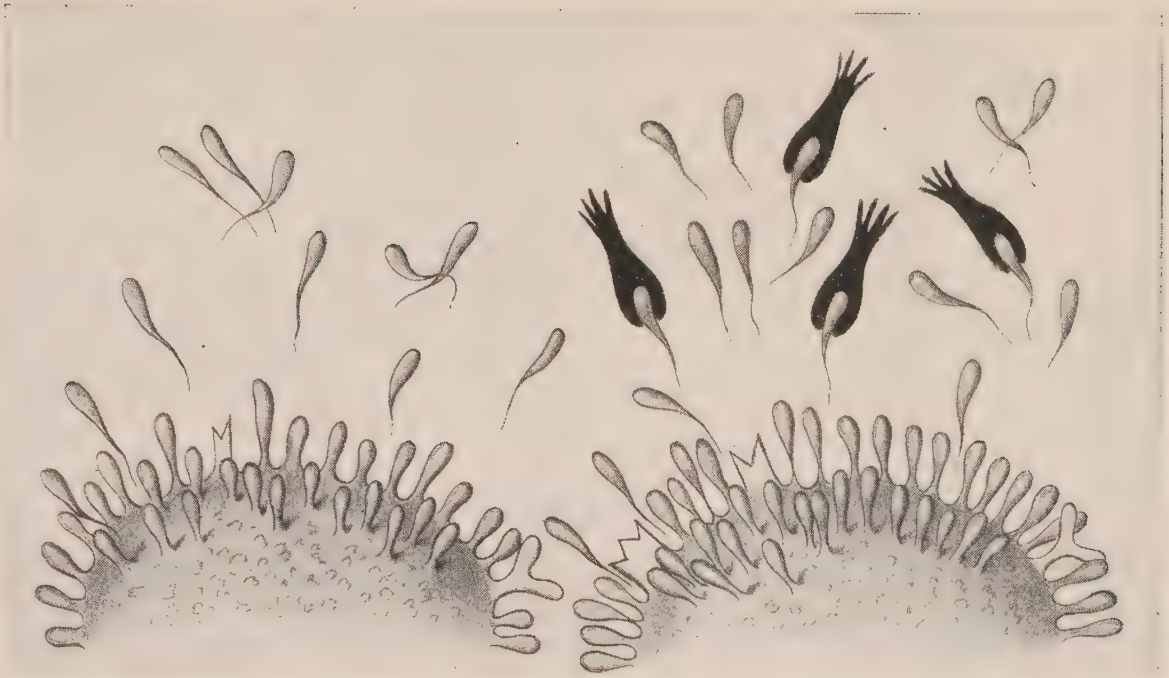


FIG. 9.—FOURTH STAGE: ANTITOXIN FREE IN THE BLOOD.

FIG. 10.—THE FREE RECEPTORS (ANTITOXIN HAVE UNITED WITH THE HAPTOPHORE WITHOUT TOUCHING THE CELL.

This diagram shows how toxin may be neutralised by antitoxin in the blood.

(*After Ehrlich.*)

the animal acquire immunity against that toxin, but its **blood-serum** will neutralise or render inert the latter if mixed with it; consequently, if we inject some of this **immune serum** into an animal already suffering from an attack of the disease caused by that particular toxin, it may enable it to recover.

We see, therefore, that 'the serum of an animal immunised against the toxins of a particular organism contains a body capable of directly neutralising the toxins of that organism, not only within the system of the immunised animal but also outside it.' This body is called an **antitoxin.**

Corresponding to the theory that normal cellular protoplasm is built up of two groups of molecules (central and lateral), so it is assumed that a **toxin** molecule also possesses two groups:

1. A stable **haptophore** group which joins on to the unstable group in the animal cell (the receptors or lateral chains), or to the antitoxin (cast off lateral chains) if such be circulating in the animal system.

2. An unstable **toxophore** group—the actual carrier of the poison—which, **as soon as the former (haptophore) group has joined on to the animal cell,** comes into action, and is now enabled to exert its toxic properties on the cell.

The toxophore group is unstable, and much more easily destroyed than the haptophore group.

A poison molecule which through any cause has lost its toxophore group is termed **toxoid** (see Fig. 12).

'Toxoids may be produced spontaneously in old poisons through decomposition of the poison molecule, or they may be produced artificially by causing certain destructive agents, such as heat or chemicals, to act on bacterial poisons' (Wassermann).

It must be noted, that a toxoid still possesses the

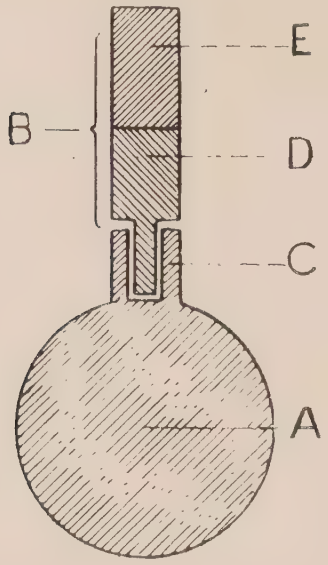


FIG. 11.—AFTER LEVADITI.

A, Cell ; B, toxin molecule ; C, receptor of cell ; D, haptophore group of toxin ; E, toxophore group.

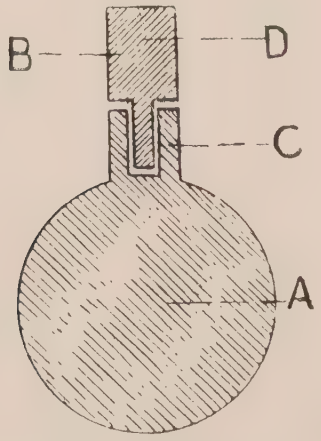


FIG. 12.—AFTER LEVADITI.

A, Animal cell ; B, toxoid—toxin molecule having lost its toxophore group ; C, receptor of cell ; D, haptophore group.

haptophore (combining) group, and is consequently still capable of combining with receptors of the body cells; but it is now less harmful to these: it no longer carries poison; it has lost its toxophore group. Yet it is capable of causing *some* damage to the cell, and consequent over-production of side chains, and for this reason toxoids may be used to immunise animals and to produce antitoxins.

The combination of the haptophore group of the toxin with side chains (receptors) of the animal cell results in damage to the latter, but the side chains alone may be destroyed, whilst the cell substance itself forms new lateral chains **in excess** of the original number, and consequently now possesses a larger number of side chains, enabling it to withstand larger and larger doses of the toxin.

Finally, so many side chains are formed that many are thrust off from the cell into the lymph and blood stream (Figs. 8, 9, and 10).

These free receptors or side chains are the antitoxin, and they can unite with toxin *before* it reaches the cell, and so protect the latter (Fig. 10).

If injected into the body of another animal they fulfil the same function there, and will protect the animal so injected against subsequent doses of toxin. Should the toxin already exist in the blood of an animal before receiving the antitoxin the latter may still neutralise any toxin which is free and uncombined with the cells, provided always that it has not become united to the *central* group of the animal cell in addition to the lateral chains; once this has happened no dose of antitoxin can be of any avail. If, however, the toxin has only combined with the lateral chains, and a sufficient length of time has not elapsed for it to combine with the central group, then injection of *large* doses of antitoxin *may* withdraw some portion of the poison from the system and enable the animal to recover.

Immunity to a toxin may be due to—

1. The absence of appropriate receptors for the haptophore group of the toxin; and, as we have already seen, the toxophore group can only act when the haptophore is properly joined to the animal cell.

Example.—Tetanus toxin, if injected into the tortoise, is harmless, but if the blood of the tortoise so injected be inoculated into a mouse, the latter dies of tetanus. Evidently the tortoise has no suitable receptors for tetano-toxin.

2. The presence in the animal's serum of antitoxin, either normally existing there or formed as the result of injecting small doses of toxin.

3. A previous attack of the disease.

4. The injection of antitoxic serum from another immunised animal.

The passive immunity resulting from injection of serum from an immunised animal is only of short duration; the antitoxin is soon excreted, and the immunity then disappears.

The above-mentioned theories apply to intoxication diseases—diseases produced by absorption of toxins, whilst the causal organism remains localised and does not itself enter the circulation. Tetanus is an example of such a disease; the bacilli remain localised to the wound, where they manufacture a poison—tetano-toxin; this is absorbed, travels along the nerves to the central nervous system, and produces the disease tetanus.

But Ehrlich does not limit his lateral chain theory only to the explanation of immunity against toxins: he applies the same theories to account for immunity to all other substances. He holds that if any substance, whether toxin, ferment, bacteria, animal cells, fluids, etc., possesses the power by means of a fitting haptophore group to combine with side chains (receptors) of the living organism, the possibility for overproduction and throwing off of these receptors is

FIG. 13.—DIAGRAM TO REPRESENT THE AMBOCEPTOR OR IMMUNE BODY LINKED WITH THE ALEXIN OR COMPLEMENT ON THE ONE HAND, AND THE RECEPTOR OF A CELL (IN THE CASE OF CYTOLYTIC SERA), OR A RED BLOOD-CORPUSCLE (IN HÆMOLYTIC SERA), OR A MICROBE (IN ANTIMICROBIC SERA), ON THE OTHER. (*After Ehrlich.*)

FIG. 14.—AN AMBOCEPTOR HAVING TWO AFFINITIES.

given—*i.e.*, the possibility to produce a corresponding *anti*-body.

Specific antibodies in the serum as a result of immunising processes can only be produced, therefore, by substances which possess a haptophore group, and which in consequence are able to form a firm union with a definite part of the living organism—the receptor.

This is not the case with alkaloids—*e.g.*, morphin, strychnine, etc., which, according to Ehrlich, enter into a loose union, a kind of solid solution, with the cells. It is for this reason that we are unable to produce any antibodies in blood-serum against these poisons (Wassermann).

Ehrlich's Theory as applied to Antimicrobic Sera.

In cholera and typhoid fever of man, pyæmia, and other diseases, the toxins appear to be intracellular (confined to the interior of the bacterial cells, and not distributed outside them).

Ehrlich's explanation of immunity in such diseases is somewhat similar to that already described under antitoxic immunity; but in the case of the diseases now under consideration the immunity is *antibacterial* rather than antitoxic—*i.e.*, the body fluids of immune animals *destroy the bacilli themselves* instead of simply rendering inert their toxins.

It must be noted that a serum may be perfectly antitoxic, and yet have no antimicrobic action. Diphtheria bacilli (man) will grow readily in diphtheria antitoxic serum.

Pfeiffer's Reaction.

Pfeiffer showed that by immunising a guinea-pig against cholera vibriones by injecting it with small and gradually increasing quantities of that microbe, the vibriones of cholera

subsequently injected into the peritoneal cavity of such an animal are rapidly killed and dissolved. Further, if some of the peritoneal fluid from a guinea-pig so immunised be added to a culture of cholera vibriones in a test-tube, the same process of destruction occurs.

If, however, before adding the immune serum to the culture we heat the former to 55° C., or allow it to stand eight or ten days before use, no such destruction occurs; but it can be again reproduced by adding a small quantity of fresh serum from any ordinary non-immunised guinea-pig.

We see by these experiments that destruction of micro-organisms is brought about by the action of two separate bodies.

1. One present in the guinea-pig before it became immunised. This substance is destroyed by heat, and also spontaneously when kept outside the body, and is known as

Alexin (Buchner),
Complement (Ehrlich), or
Cytasis (Metschnikoff).

2. Another body produced only in the immunised animal, and resistant to heat; this is termed

Amboceptor (Ehrlich),
Fixateur (Metschnikoff),
Immune body,
Sensibilitising substance,
Copula, or
Desmon.

Ehrlich explains the solvent action of antimicrobic sera on bacteria by his lateral chain theory in the following manner:

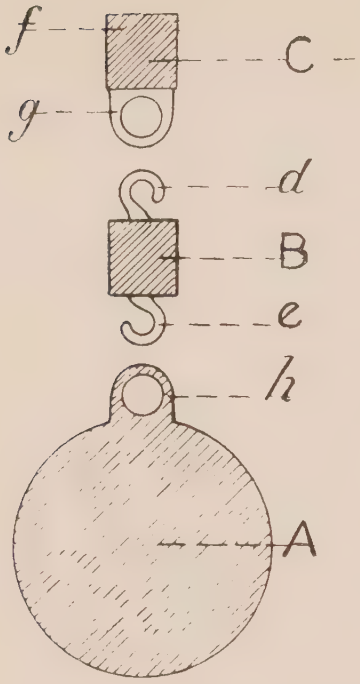


FIG. 15.—ANTIBACTERIAL AND HÆMOLYTIC SERA.
(AFTER LEVADITI.)

A, in the case of a hæmolytic serum, would represent a red blood-corpuscle, or, in the case of an antimicrobic serum, a microbe; B, amboceptor; *d*, complementophile group; *e*, cytophile group; C, complement (alexin); *f*, its zymotoxic group; *g*, its haptophore group.

In this form of immunity—*i.e.*, under the repeated injection of **bacteria** (not toxin alone, as in the production of antitoxic immunity)—the side chains produced in excess by the body cells are cast off, and receive the name of **amboceptor** (or immune body, fixateur, etc.).

The **amboceptor** is composed of two groups—

1. A cytophile group.
2. A complementophile group.

The amboceptor joins on to the bacteria in the following manner: Its cytophile group (Fig. 15, *e*) joins on to a

bacterium, whilst its complementophile group unites to the complement (alexin), and this latter acts as a ferment, and is now enabled to digest or destroy the bacterium, linked to it as it is by means of the amboceptor.

The complement or alexin (normally present in the blood-serum of animals) cannot *by itself* unite with bacteria, perhaps because the side chains of the two do not 'fit' each other.

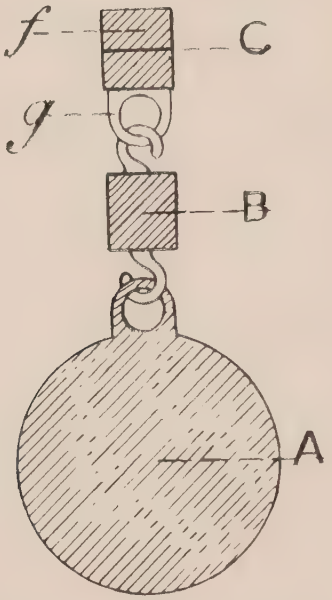


FIG. 16.—AFTER LEVADITI.

A, Micro-organism or, in the case of hæmolytic serum, a red blood-corpuscle; B, immune body or amboceptor; C, complement.

The amboceptor, however, possesses side chains 'fitting' and capable of combining with *both* the side chains of bacteria and the alexin; it serves, in fact, as a coupling link between the alexin and the microbe.

Only after the amboceptor has united with the bacteria can the dissolving substance (alexin or complement) unite with it and begin its solvent action.

Exactly in the same way that a toxin is assumed to be built up of two groups of molecules—haptophoric and toxophoric (see p. 7)—so the **complement** or alexin is also supposed to have two groups: (1) A combining **haptophore** group (see Figs. 15 and 16, *g*), which *withstands* heating to 55° C.; and another group (2) much more fragile, the **zymotoxic** group (Figs. 15 and 16, *f*)—it is this group which possesses the actual solvent properties of the complement.

Just as toxins which have lost their toxophore group are called toxoids, so complements which have lost their zymotoxic group are termed **complementoids.**

To recapitulate, we see that only after the haptophore group of the complement has united with the complementophile group of the amboceptor, and the latter by means of its cytophile group has united with the receptor of the micro-organism, can the zymotoxic group of the complement begin its solvent action on the bacterium indirectly linked to it.

Ehrlich believes, then, that active immunity against bacteria is due to the co-operation of the two substances, amboceptor and complement.

Bordet's explanation of the way in which the amboceptor or immune body (or the *substance sensibilitrice*, as he prefers to term it) acts differs somewhat from Ehrlich's views, inasmuch as he assumes that it acts as a **mordant** on the bacteria (in antimicrobic sera) or red blood-corpuscles (in hæmolytic sera), **sensitising them** to the action of the alexin. He considers that there is only one single complement or alexin in the serum, and this is capable either of dissolving red blood-corpuscles or bacteria once they have been **sensitised** by their specific immune body.

Now, Ehrlich believes in the multiplicity of complements; for instance, that the complement which fits to the bacterial

immune body is different from that for the hæmolytic immune body, and he and other savants, by many beautiful experiments, have confirmed this belief.

In addition to the purely cellular or purely humoral theories of immunity, there is still another type—

The Opsonic Theory

of Wright—and this appears to be a combination of both humoral and cellular theories.

In some cases it has been found that the serum of an animal immune to a particular micro-organism is yet devoid of bacteriolytic power, but, instead of itself dissolving the bacteria, it is possessed of the power of so acting on them that the phagocytes are enabled to take them up, digest and remove them with facility.

This peculiarity is seen in relapsing fever (human), pneumonia, infections with staphylococci, tubercle bacilli, and probably many other organisms.

To the substances formed in the blood serum of animals, and which enable the leucocytes to attack the bacteria, Wright has given the name of

Opsonins (' Feast-preparers ').

Wright and Douglas agree with Metschnikoff that the final destruction of microbes is effected by the phagocytes, but they affirm that leucocytes are practically unable to pick up microbes by themselves; they can only do so after the micro-organisms have been impregnated with opsonin, and so rendered suitable prey for the phagocytes.

During the process of active immunisation they find that the **opsonic value** of a serum is increased—*i.e.*, an increased amount of opsonin is formed therein.

A number of experiments were made by these two

workers, and these have been cited in proof of the 'opsonic theory.'

The action of normal serum, microbes, and phagocytes was studied experimentally outside the body :—

1. When normal unheated serum, phagocytes, and bacteria were mixed together, phagocytosis promptly appeared.

2. By making use of serum which had been heated previously to 60° C. for fifteen minutes (to destroy the opsonin dissolved therein), phagocytosis was delayed or prevented.

3. But it was found that when unheated serum was mixed first with bacteria at 37° C. for fifteen minutes, and *then* heated to 60° C. for the same length of time, on again reducing the temperature to 37° C., and adding washed leucocytes, phagocytosis readily occurred. Evidently in this case the opsonin had completed its task before destruction.

As to the exact *nature* of opsonins there is some doubt. They are quite distinct from Buchner's alexins; in action they resemble rather an amboceptor.

According to Metschnikoff (Harben Lectures, 1906), 'the question whether in the serum of normal and of immunised animals there exists one single substance which can fix itself on to the microbes, or whether there are two different substances, opsonin and sensitising or fixing substance (see pp. 26 and 29), has given rise to several very delicate researches, which have not yet led to a definite solution. According to the opinion of Dean, of the Lister Institute — an opinion which seems most probable to us—in all these substances of the body-liquids we have only to do with sensitising substances.

'The fact that normal sera after being heated to 60° C. no more favour phagocytosis, whilst the sera of immunised animals preserve the power in spite of heating, might, according to Dean, be explained by differences in the quantities of the contained sensitising substances. The

normal sera, which only contain a small amount of them, lose their activity after a short heating to 60° C., whilst the sera of immunised animals, being ever so much richer in sensitising substances, retain a sufficient quantity of them even under such conditions, and do not lose them completely until they are heated to between 65° and 70° C.'

If the opsonin is really essential to phagocytosis, whether it be identical with or separate from the sensitising substance (*fixateur*, see p. 29), Metschnikoff considers that it originates from the leucocytes, and from these it finds its way into the serum.

This savant isolated leucocytes from the peritoneal exudate of guinea-pigs vaccinated against anthrax. These were first washed and then emulsified in normal saline solution, and anthrax bacilli added. Phagocytosis occurred promptly, here evidently without the presence of serum opsonin, for he added none of this body, and serum was not used in the experiment. He says: 'Either the absorption of the microbes may be effected without the help of the opsonin, or, should such help be indispensable, the opsonin may be supplied by the leucocyte itself.'

So that Metschnikoff, notwithstanding the theories ascribing to phagocytes but a secondary rôle in the process of immunity, still maintains that the phagocytes are the essential factors. 'It is the phagocytes,' he says, 'which deliver us from our enemies.'

These, then, are the principal theories held at the present day regarding the nature of immunity against microbic infection and intoxication. But not only are *bacteria* destroyed within the animal economy, exactly the same happens if the blood-corpuscles of one species of animal are injected into an animal of different species (**hæmolysis**), or if cells obtained by macerating the tissues of one species of

animal be injected into the circulation of another (**cytolysis**).

Example.—If we inject erythrocytes from a horse into the vascular system of a dog, the latter animal will produce a serum capable of dissolving the red blood-corpuscles of *any* horse, and if injected into the circulation of any equine, it would cause dissolution of erythrocytes, symptoms of hæmoglobinuria, and perhaps death.

Such a serum is termed **hæmolytic**, or if, instead of using red blood-corpuscles, we make use of other cells of the body, then the serum would be known as **cytolytic**.

These sera act exactly in the same manner as the antimicrobic previously described, and the destruction of the foreign cell is brought about by the co-operation of alexin and amboceptor (see Figs. 15 and 16).

Agglutination.

When bacteria are injected into animals, in addition to the bacteriolysins formed in that animal's serum, another body becomes prominent therein, which agglutinates or causes coherence of the organisms, so that if some blood-serum from such an animal be added to a corresponding culture of the bacteria in a test-tube, the microbes collect in flocculi, and finally fall to the bottom

The same is true for hæmolytic sera, hæmagglutinins as well as hæmolysins being formed in these sera.

Agglutinins are distinct and quite separate from the lysins; they may be formed in a serum at the same time, but are not identical. There are sera which dissolve certain cells without agglutinating them, and others which agglutinate cells without dissolving them.

Normal blood-serum possesses *some* power of agglutinating or clumping foreign organisms or cells, and also *some*

bacteriolytic and cytolytic action, but these properties are enormously increased in the natural or artificial infection of an organism with foreign elements.

Substances formed in the body which bring about this agglutinative reaction are known as **agglutinins.**

According to Wassermann, 'the agglutinins are fairly resistant substances, which withstand heating to 60° C., and lose their power only on heating to 65° C. It is possible, therefore, to make a serum hæmolytically inactive by heating it to 55° C., and still preserve its agglutinating power.

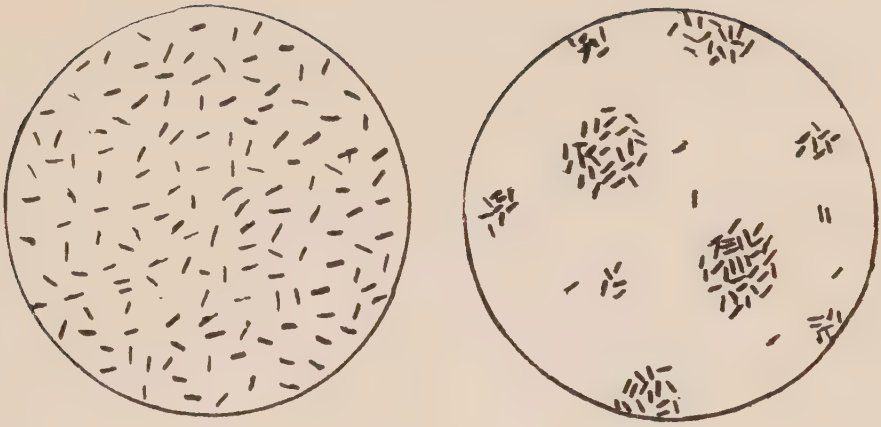
'Corresponding to the specific combining-power of these agglutinins, they possess a haptophore group which effects the combination, and a second **agglutinophore** group—easily decomposed by acids—which effects the clumping.'

Agglutinins which have lost their agglutinophore group, but which still possess their haptophore group, are called **agglutinoids**—just as toxins which have lost their toxophore group are called toxoids, or complements which have lost their zymotoxic group are called complementoids.

Such agglutinoids may still combine with bacteria or foreign cells by means of their haptophore group, but they will not be able to produce any clumping or agglutination, having lost their agglutinophore group.

The reaction (agglutination) is used for diagnostic purposes. Gruber first called attention to the fact that the blood-serum of patients suffering or convalescent from typhoid fever, when added to a bouillon culture of the *Bacillus typhosus*, possesses the power of causing the bacilli to adhere together in clumps and to lose their motility—in other words, to become agglutinated (Figs. 17 and 18).

Grünbaum, it is said, first applied the principle of this agglutinative reaction to the diagnosis of typhoid fever, but



1. 2.

FIG. 17.—DIAGRAM ILLUSTRATING AGGLUTINATION OF BACTERIA.

1. Uniform suspension of bacteria—organisms evenly distributed.
2. The same after addition of an agglutinating serum.

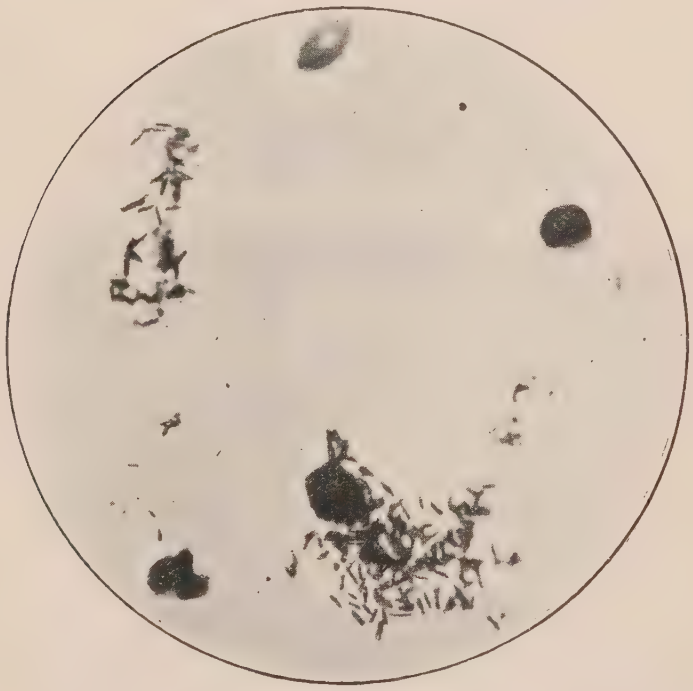


FIG. 18.—AGGLUTINATION OF BACILLUS TYPHOSUS BY BLOOD-SERUM FROM A TYPHOID PATIENT (MICROPHOTOGRAPH).

he was anticipated in his publication by Widal; hence Widal's name is usually associated with this method of diagnosis in the case of the disease mentioned.

The principle (Widal's serum reaction) has since been applied to the diagnosis of a number of animal diseases, notably glanders (see p. 163).

The agglutination reaction is also utilised by the bacteriologist in differentiating various micro-organisms.

Example.—Fowl cholera and swine plague. These diseases belong to the group known as the animal Pasteurelloses, and the causal agent of both presents practically identical microscopical and cultural features; yet, according to Jess, serum from a fowl cholera immune animal agglutinates only fowl cholera cultures, and has no action on those of swine plague.

Again, organisms are encountered bearing a very close resemblance to the *B. typhosus*, and are only distinguished therefrom by their failure to agglutinate in the presence of serum from a typhoid patient. In such cases a valuable and delicate test is furnished by agglutination.

Precipitation.

If we inject the serum of one species of animal into an animal of a *different* species, the serum of the latter gains the power of producing a precipitate when added to the serum of any individual of the former species.

Example 1.—If we inject the blood-serum of a man into a rabbit, the serum of that animal will produce a precipitate when added to *any* human serum.

The substances produced in the animal so injected, and which bring about this reaction, are known as **præcipitins.** We distinguish **sero- and musculo-præcipitins** according to whether the serum has acquired its precipitating activity

in response to the injection of blood-serum or muscle extract.

Example 2.—If, after macerating a quantity of minced horse-flesh with distilled water, we filter it, and inject the fluid so obtained into an animal of a different species (rabbit, dog, etc.), that animal will furnish a serum capable of giving a precipitate with any aqueous extract of horse-flesh.

Instead of horse-flesh we may substitute that of oxen, sheep, goats, pig, dog, cat, etc., taking care to use an animal of quite a different species for the purpose of experiment; in every case the animal injected with such fluid will produce a serum giving a precipitate when added to an extract of muscle from any individual of the species from which it was injected.

Example 3.—Milk may be coagulated by adding to it the blood-serum of an animal into the circulation of which milk had previously been injected. Such a serum was termed by Bordet a *lacto-serum.*

In nature præcipitins resemble agglutinins, consisting of a combining or haptophore, and a second group which acts through the former, bringing about the reaction, precipitation. Præcipitins are, in fact, another variety of cast-off receptor produced by the animal cell, resulting from the injection of **dissolved** foreign albuminous substances.

Precipitation is used to detect the blood and flesh of different animals, the medico-legal identification of blood-stains, and the adulteration of sausages, etc., with horse, dog, cat, or other flesh.

Experiments conducted to test the value of this new method of diagnosis by Von Rigler, Nicholas, Vallée, and others, have given satisfactory results (see pp. 169-173).

Regarding the value of the test in the identification of blood-stains, Halliburton makes the following statement:

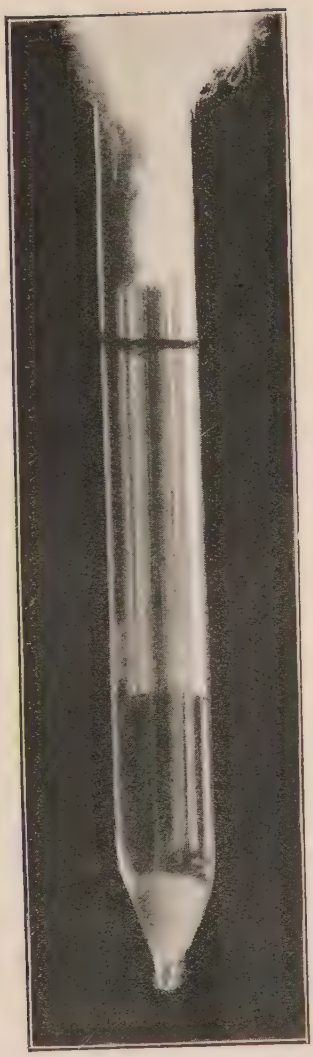


FIG. 19.—PHOTOGRAPH OF TUBE CONTAINING A MIXTURE OF HORSE BLOOD-SERUM AND THE SERUM OF A RABBIT WHICH HAD PREVIOUSLY RECEIVED PERIODICAL INJECTIONS (EIGHT, AT INTERVALS OF THREE DAYS) OF HORSE SERUM.

A well-marked precipitum is shown.

FIG. 20.—PHOTOGRAPH OF TUBE CONTAINING A MIXTURE OF OX BLOOD-SERUM AND THE SERUM OF A RABBIT PREVIOUSLY INJECTED WITH HORSE SERUM (THE SAME RABBIT USED IN PREVIOUS EXPERIMENT, FIG. 19).

In this case there is *no* precipitum.

'To discover whether the stain is blood or not is by no means a difficult problem; but to distinguish human blood from that of the common mammals is possible only by the biological (præcipitin) test.

'The great value of the test is its delicacy; it will detect the specific blood when it is greatly diluted, after it has been dried for weeks, or even when it is mixed with the blood of other animals.'

In the examination of old blood-stains on clothing it is, of course, necessary to dissolve the dried blood in physiological salt solution before adding the antiserum.

CHAPTER II

THE METHODS OF CONFERRING IMMUNITY

The methods of producing immunity—Monovalent and polyvalent vaccines and sera—The Pasteurelloses—Fowl cholera—Canine distemper—White scour—Antistreptococcic serum—Anthrax—Tetanus—Diphtheria antitoxin—Black quarter—Louping ill and braxy—Tuberculosis—Artificial immunisation of cattle against tuberculosis — Glanders — Swine erysipelas — Swine plague—Swine fever.

Methods of producing Active Immunity.

A. A pure virus may be injected into the animal to be immunised, but, to prevent a fatal issue, this is introduced in small doses, and in a manner differing somewhat from that in which the virus naturally gains access to the body. Example : pleuro-pneumonia.

B. An attenuated (weakened) virus may be injected.[1] The virus may be attenuated—

1. By drying. Example : rabies.

2. By heat. Example : black quarter.

3. By passing it through other animals. Example : swine erysipelas.

4. By the addition of chemical agents. Example : the immunisation of horses against the toxins of tetanus for

[1] An attenuated virus which, when introduced into the body of an animal, enables it to withstand the development of an infective disease is known as a vaccine or a virus-vaccine.

the production of an antiserum. In this case a solution of iodine may be used.

5. By prolonged cultivation in artificial media. Example: fowl cholera.

6. By attenuation from unknown causes in the bodies of sick or recovered animals. Example: rinderpest.

C. Sero-virus Inoculation or Sero-vaccination.—In this method both antiserum and virus are injected; the immune serum 'mobilises the defences of the body, and enables the animal to receive a feebly attenuated or even normal virus, which confers a durable immunity.'

The serum and virus may be introduced in four different ways:

1. The virus may be injected first and the serum soon afterwards.

2. Serum first, followed by virus.

3. Both injected practically at the same time, but at different points or different sides of the body.

4. A mixture of serum and virus may be injected.

Example of sero-virus vaccination: swine erysipelas, rinderpest.

The method of conferring a passive immunity on animals is by the injection of blood-serum taken from an animal actively immune against the disease in question.

The Advantages respectively of Active and Passive Immunisation.

Leclainche gives the following '**indications for the production of a passive immunity by means of serum**:

'1. To prevent a temporary threatening of infection, as in tetanus, or to protect subjects momentarily exposed to contagion, as in fairs, shows, etc.

'At the commencement of epizootics, so as to circumscribe the primary centres, as in cattle plague (rinderpest), foot-and-mouth disease, sheep pox, and to prevent the costly measure of general slaughter.

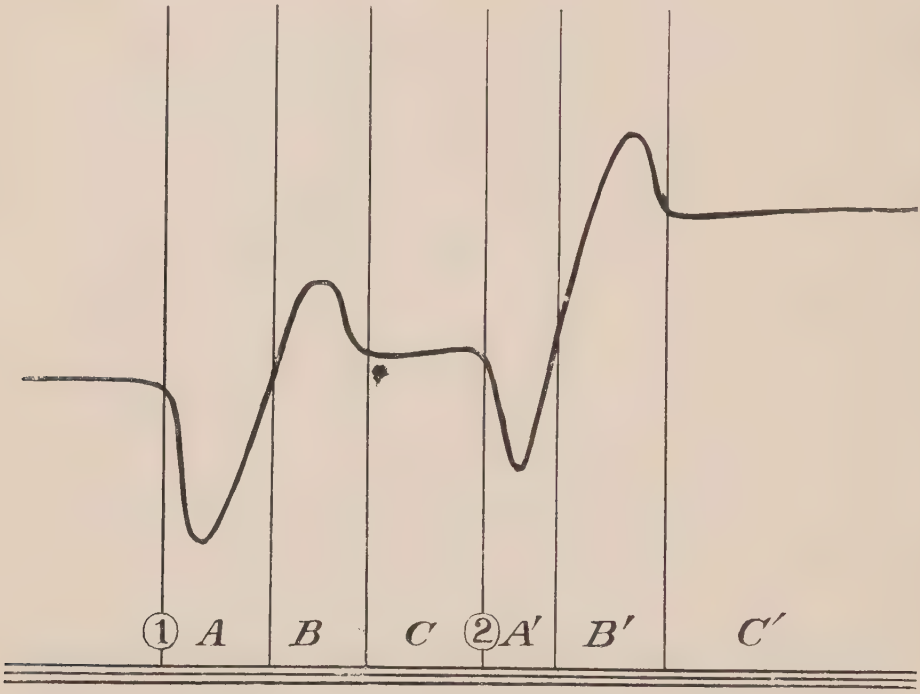


FIG. 21.—DIAGRAM ILLUSTRATING THE PROCESS OF ACTIVE IMMUNISATION.

'1' corresponds with injection of first vaccine, and '2' with second vaccine.

A and *A′*, Negative phase—lowering of defensive powers and increased susceptibility to infection; *B* and *B′*, recovery—positive phase; *C* and *C′*, higher base-line of immunity: at *C′* immunity is firmly established.

'3. To protect the whole stud, so that it may be kept free for some time, as in cases of urgent work, cavalry, etc.

'4. To save the contaminated in affected surroundings and districts from a rapidly spreading contagion, as in swine erysipelas, fowl cholera, etc.'

We have seen (p. 4) that in the process of active immunisation the immunity is but slowly acquired. It is not firmly established until some time after the injection of attenuated virus (vaccine).

The first and immediate effect of vaccination is to lower the animal's defensive powers and increase its *susceptibility* to infection. This is known as the **negative phase** of immunisation.

During the continuance of this phase the available side chains of the body cells have probably joined on to the virus, and the cells are being stimulated or educated to withstand its presence, but sufficient time has not elapsed for an excess of side chains to be formed and cast off.

Following on the negative occurs the **positive phase**, in which the body has acquired some resistance to infection.

When large doses of vaccine are injected into an animal, the negative phase is very marked, and may last for weeks. On the other hand, when small doses are given, the negative phase is only slight, and is soon succeeded by the positive phase.

In view of the fact that during the continuance of the negative phase the animal is especially liable to infection, it is better and safer to administer two or more small doses of vaccine rather than one large one.

A sufficient length of time must elapse between each injection, so that the patient may entirely recover from the effects of one dose before being subjected to a second.

The animal must be guarded against all sources of infection throughout the negative phase. In the presence of an epizootic, and lacking means of strict isolation, there is considerable risk in attempting to confer active immunity.

Under such conditions it is advisable to adopt a preliminary passive immunising process by injecting serum from an animal previously immunised. In this way an

immediate resistance is conferred against infection, and, before such temporary protective influence has passed away, by injecting virus-vaccine it is possible with safety to change this temporary passive immunity into one more lasting—into an active immunity.

Monovalent and Polyvalent Vaccines and Sera.

It has been found from experience that different *strains* of a particular species of micro-organism are frequently of different degrees of virulence.

A monovalent serum is prepared by injecting into the animal which is to furnish the serum *one strain only* of a particular organism.

A polyvalent serum is prepared by injecting into the animal which is to furnish the serum *several different strains* of the *same species* of microbe.

Example.—Marmorek's antistreptococcic serum.

Marmorek found that the pathogenicity of the *Streptococcus pyogenes* could be greatly increased by growing it alternately in the peritoneal cavity of the rabbit and in a mixture of human serum and bouillon, until one or two microbes constitute a fatal dose.

After about twenty or thirty passages through the rabbit the virus attained a fixed virulence—*i.e.*, it attained its maximum virulence.

He found that an active serum could be prepared by treating horses with this fixed virus. This serum was, of course, only a monovalent one—*i.e.*, it was active only against the one strain of the particular organism, *Streptococcus pyogenes*.

Later he obtained a polyvalent serum by making use of many different streptococci from both men and animals.

A monovalent and polyvalent vaccine may be prepared by utilising respectively either a single strain or many different strains of a particular organism.

The polyvalent vaccine of Lignières is composed of a *mixed* culture of pasteurella organisms obtained from the horse, ox, sheep, pig, dog, and fowl, and a vaccine so prepared should be active against the pasteurelloses of any or all of these animals.

A monovalent vaccine would, of course, be prepared (in the case of the pasteurella) from, say, the organism causing fowl cholera alone; this vaccine would be useless against any of the pasteurelloses of animals other than the fowl.

The Pasteurelloses,

also known as the 'hæmorrhagic septicæmias,' are a group of diseases affecting many different species of animals, but these maladies in all show a great similarity, and the causal agent in each case presents the general characteristics of the pasteurella organism, of which the fowl-cholera bacillus forms a typical representative.

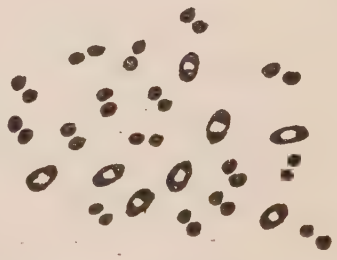


FIG. 22.—FOWL-CHOLERA BACILLI.

Small ovoid bacteria, showing bipolar staining, appear as figure-of-eight or as 'vacuole' bacilli according to intensity of staining.

The pasteurella organism is a cocco-bacillus, non-motile, polymorphous, does not stain by Gram's method, does not form spores. Aerobic, but can be grown as an anaerobe, and when injected into the blood-stream the organisms show a predilection for serous and synovial membranes.

List of the Animal Pasteurelloses.

1. **Horses.**—Influenza, 'typhoid fever,' contagious pneumonia.
2. **Cattle.**—Septic pleuro-pneumonia of calves, pneumo-enteritis or cornstalk disease, entoque (Brazil), diarrhœa (white scour) of calves.
3. **Wild Animals.**—Pneumo-enteritis, wild seuche.
4. **Buffalo.**—Barboné.
5. **Goats.**—Infectious pneumonia (India and Africa).
6. **Sheep.**—Pneumo-enteritis, lombriz.
7. **Swine.**—Swine plague or contagious pneumonia.
8. **Dog.**—Distemper, canine typhus.
9. **Cat.**—Distemper, broncho-pneumonia (Siam).
10. **Birds.**—Fowl cholera, avian diphtheria.
11. **Rabbit.**—Septicæmia, epizootic rhinitis.
12. **Guinea-pig.**—Septicæmia.

White Scour in Calves; Distemper of Dogs and Cats.

These two diseases are still included in the list of the animal pasteurelloses, but, according to recent investigations, it is doubtful whether the pasteurella organism is here the true etiological factor.

Jensen considers that white scour is due to organisms of the coli type, whilst according to Carré canine and feline distemper result from infection with an ultravisible virus.

The pasteurella organisms are universally distributed, occurring in food and water, soil, and in the respiratory and digestive tracts of the body.

Saprophytic ordinarily, they are capable of becoming parasitic and infecting the body, and although alone they may give rise to no specific symptoms, yet they reduce the natural resisting powers of the animal system, and

in this way **pave the way for secondary infection** by other organisms, some of which are commonly found in the body, and under ordinary conditions are harmless, but after the preliminary action of the pasteurella are enabled to acquire pathogenic properties and become extremely harmful.

Lignières considers in the case of the horse—catarrh, laryngitis, influenza, strangles, contagious pneumonia—there is but one causal agent, viz., the pasteurella, supplemented in the case of catarrh, strangles, and pneumonia by a streptococcus identical in all these diseases.

Purpura is also included in the diseases in which the pasteurella may pave the way for other organisms. (Cadeac 'supposes that any toxin causing capillary dilatation may determine the disease (purpura), and calls attention to the potent vasodilator action of the products of strangles, contagious pneumonia, and influenza, which are among the most frequent antecedents of petechial fever'—Law).

We see that *primarily* many different diseases of animals may be ascribed to the pasteurella organism; a vaccine against these organisms should therefore be of value in veterinary practice. Such a vaccine was introduced by Lignières, an extract from whose report to the French Academy of Science follows:

In the preparation of

Lignière's Polyvalent Vaccine against the Pasteurelloses

'the **mixed** cultures (from all the pasteurelloses of animals) were kept up on gelose agar by reinsemination every two days, and only those cultures were used that were thus maintained for over a year, and at the present time all our cultures have been removed for more than 500

times, as we believe by this to be able to more surely prevent the always formidable virulence from returning.

'To prepare the vaccines these cultures are inseminated in flat-bottomed flasks containing a layer of bouillon peptone of 1 to 2 centimetres thick; these flasks are afterwards kept in a temperature of 42° to 43° C. for five days for the **first vaccine**, and two days for the **second vaccine**.

'The attenuation obtained by these means allows all species of animals of any age to be safely inoculated.'

Dose:

Horse or ox	$\frac{3}{4}$ to 1·3 c.c.
Calf or pig	$\frac{1}{2}$ c.c.
Sheep or medium-sized dog ...	$\frac{1}{4}$ c.c.
Rabbit or fowl	$\frac{1}{8}$ c.c.

Two vaccinations should be given, the second with the stronger vaccine twelve to fifteen days after the first.

The injections are made either subcutaneously or intraperitoneally.

In Lignières' experiments the mortality did not exceed 12 to 15 per cent., whereas it was 50 per cent. for the control animals. He advises that in any locality where the disease permanently occurs it is an indication for the animals to be vaccinated eight to ten days after their birth.

Practically, immunity conferred by inoculation should be considered as lasting no longer than a year. It is advisable, therefore, to revaccinate every year without waiting for a return of disease.

A preventive and curative polyvalent serum has also been prepared by Lignières.

The polyvalent vaccine is inoculated into horses in small doses, repeated at intervals of a few days. At first the injections are given subcutaneously, but when the animal has acquired a certain degree of tolerance they are given intravenously.

The blood-serum of animals immunised in this way, when used early in cases of canine distemper, equine influenza, pneumonia, etc., has given good results, but it has no action in the case of secondary infections following or complicating the pasteurella.

Dose for the dog subcutaneously is 15 to 30 c.c.; intravenous, 5 to 10 c.c.

Fowl Cholera.

Pasteur's vaccines are obtained by exposing bouillon cultures of the fowl cholera bacillus to the air for from three to ten months, attenuation of the virus depending upon the length of time so exposed.

Inoculation of the first vaccine produces only slight illness, with prompt recovery. The second stronger vaccine should be used ten or twelve days after the first. This gives an active immunity.

The drawbacks to this method of inoculation are summarised by Kitt:

1. Fowls are usually of too little value to warrant inoculation.

2. In infected flocks where it is employed the more susceptible birds are usually already contaminated, and a large proportion die in spite of it.

3. It becomes a means of planting the infection in new localities.

Kitt has shown that it is possible to obtain from horses by subcutaneously inoculating them with virulent cultures of the *Bacillus avisepticus* (fowl-cholera bacillus) a **serum** capable of giving to certain animals an appreciable resistance to fowl cholera.

The horses were inoculated with gradually increasing doses of virulent cultures of from $\frac{1}{2}$ to 10 c.c. at intervals of four to five days, and they furnished a serum capable of

protecting small animals in comparatively small doses (2 to 5 c.c.).

For the goose, duck, fowl, and rabbit the serum may serve to *protect*, in the course of an epizootic, animals not yet infected, but it has *no curative* action.

After inoculation immunity only lasts about eighteen days.

The inoculation of virulent material, practised simultaneously or consecutively to the immunising process, gave no useful practical results—*i.e.*, did not confer active immunity.

Lignières' polyvalent vaccine and serum should prove of service in this disease, as in all other animal pasteurelloses (see p. 50).

Canine Distemper.

Whether canine distemper should be included in the list of animal pasteurelloses is a doubtful point. Until recently the specific microbe was described as obtainable from the blood of affected dogs during the early stages of the disease and before complicated by secondary infections. In the latter case it was stated that a pure culture could be obtained by inoculating the cerebro-spinal fluid intraperitoneally into a guinea-pig.

In the dog this organism appears as a long, thin bacillus, but in the body of the guinea-pig it quickly changes to the cocco-bacillary form of the pasteurella group.

The preparation of **Phisalix's vaccine**—based on the assumption that the bacillus described above is the true causal agent of distemper—corresponds to Pasteur's method of preparing fowl-cholera vaccine. 'It consists in producing active immunity in a dog by injecting a series of virus-vaccines of various degrees of virulence. These vaccines contain the living but attenuated microbe of the disease, and when injected produce a degree of resistance proportionate to the degree of virulence of the microbe.'

The attenuation is obtained by making cultures of the specific microbe (?) of distemper in 6 per cent. glycerine bouillon. It is progressively attenuated with the age of the culture, and by reinoculating ordinary bouillon cultures of varying degrees of virulence are obtained.

'To restore the microbe to its former virulence it is necessary to pass it afresh through the organism of the dog, and after thirty-five passages have been made through this animal an increase of such virulence is obtained that an inoculation of $\frac{1}{10}$ to $\frac{1}{5}$ c.c. of the culture into the veins suffices to kill a young dog of 40 pounds weight in a few hours.'

Phisalix's first vaccine consists of a very attenuated virus —*i.e.*, an old culture.

Fifteen to twenty-one days after injecting the first vaccine a second inoculation is performed with a stronger—*i.e.*, more virulent—culture, the second vaccine.

Dose of the Vaccines :

For puppies of from six to eight weeks old	2 c.c.
For puppies of two to three months old	$2\frac{1}{2}$ c.c.
For puppies over three months old and for adults	3 c.c.

Two inoculations suffice for the majority of cases, but where a malignant form of distemper prevails a third inoculation may be necessary, either with a larger dose of the second vaccine or by using a still stronger or only slightly attenuated culture.

Reaction.—Within a few hours there is slight fever, dulness, loss of appetite, and stiffness of the inoculated limb, especially if there is a swelling (which is to be desired) at the seat of inoculation.

It is recommended that inoculation be adopted early,

preferably at the age of two months, before the puppies are exposed to the risk of coming in contact with distempered dogs, or of being placed in infected quarters or surroundings ; but dogs of any age may be inoculated.

The disease is not likely to be prevented if the dog be exposed to contagion during the vaccination period, or is already the subject of infection at the time of inoculation.

In the foregoing account of Phisalix's vaccine, Gray's articles on the subject have been drawn upon. He has been its chief exponent in England, but investigations conducted by McFadyean, Hunting, and others, have proved that, however useful Phisalix's vaccine may be in France, it is useless for the canine distemper of this country. Perhaps the reason why this vaccine has proved worthless depends on the fact that the organism from which it is prepared is not the true causal agent of distemper at all, but only a concomitant or secondary infection.

Carré has recently shown that the serous discharges taken at the commencement of the complaint are powerfully pathogenic, and the virulence is due to an organism sufficiently small to pass through certain filters—*i.e.*, an ultra-visible virus.

Gray advises that the blood-serum derived from an animal recovered from distemper should be tried on valuable breeds, such as Japanese, which nearly always succumb when suffering from distemper. 'Distemper pure and simple,' he adds, 'is usually to be dreaded when complicated with the secondary or streptococcic infection; hence the similarity of the disease with equine strangles, and in consequence the recommendation that the blood-serum of a horse recovered from strangles be used in the treatment of distemper, or, in place of this, antistreptococcic serum may be tried.'

I think we may safely consider that up to the present

time no protective or curative inoculation methods of any value have appeared for this disease.

'White Scour,' or Diarrhœa of Young Calves.

By certain authors this disease has been classed amongst the pasteurelloses; it was considered that the pathogenic agent gained access to the animal economy solely by means of the umbilical vessels. Against this theory are the facts that the organism is seldom found in the blood-stream. Attempts at infection by the alimentary tract with cultures of the pasteurella failed even when very large doses were employed. Subcutaneous inoculation gave varying results, and even direct intravenous injection occasionally failed to produce the disease.

Jensen believes the disease to be produced by one or more of the following organisms:

1. By the *B. coli communis.*
2. By *paracoli* bacilli.
3. In rare cases by the *B. pyocyaneus* or *proteus.*

Consequently, he has utilised for the prevention and treatment of white scour—(1) a coli serum; (2) a paracoli serum.

The **coli serum** is prepared by injecting horses intravenously with bouillon cultures of the *B. coli* incubated at 37° C. for twenty-four hours.

The injections are made at intervals of ten to twelve days; a slight reaction follows, sometimes colic, fever, and anorexia, lasting a few hours. Apparently a polyvalent serum, prepared from several different strains of coli bacilli, is indicated as desirable in the treatment of white scour. Jensen therefore, in order to obtain a polyvalent serum, injected horses intravenously at intervals of fourteen days with several different strains of this organism, each strain

being cultivated separately in bouillon at 37° C. for twenty-four hours, then all the strains mixed together and injected.

He states that there appears to be no objection to beginning the immunisation with single strains, and gradually increasing the number of these latter.

A **paracoli serum** is prepared in a manner similar to the preceding, but possessing, as these organisms do, 'much stronger pathogenic properties than is the rule with the coli forms, inoculations into horses must be undertaken with great care, for an intravenous injection of 0·1 to 0·25 c.c. of a bouillon culture may determine a dangerous illness.'

Jensen advises that if one is already in possession of a paracoli serum, one should give a preliminary dose, and follow this up by the first injection of culture.

A paracoli serum is never active against an infection produced by a coli form, neither is a coli serum active against paracoli bacilli.

For the prevention and treatment of white scour, Jensen has experimented with these sera injected subcutaneously, and reports favourably thereon.

Antistreptococcic Serum.

The principal streptococcic diseases of animals are:

1. Strangles.
2. Catarrhal mastitis.
3. Peritonitis following castration.
4. Pyæmia and certain forms of septicæmia.
5. Polyarthritis in foals.
6. Rheumatoid arthritis with endocarditis in cows.
7. 'Febrile cold.' McFadyean states that the streptococcus of strangles cannot be distinguished from an organism present in the nasal discharges of severe catarrh

cases. He believes that many cases of so-called *febrile cold* are due to strangles, although not diagnosed as such.

8. We have already referred to the influence of streptococci in the causation of purpura hæmorrhagica (p. 49).

9. Lignières first proved the complete identity of the strangles streptococcus with the organism described by Schutz in equine pneumonia. He called attention to the gravity of those lesions which follow the intervention of the streptococcus in pneumonia, pleurisy, etc., whether as a

FIG. 23.—STREPTOCOCCI.

primary infection or indirectly following upon an infection by a pasteurella.

Dassonville and **De Wissocq** later studied systematically many cases of strangles, pneumonia, and pleurisy, and, *without a single exception*, always found the streptococcus of strangles in the diseased tissues, either in pure culture or as a mixed infection.

They consider, therefore, that 'in the immense majority of cases our efforts must be directed against this streptococcus if we wish to prevent the development of fatal lesions.'

These two authors further proved the identity of the strangles streptococci with the organism found in pneumonia, pleurisy, etc., by immunising horses against the strangles streptococcus by injecting them with progressively increasing doses; these horses afterwards resisted inoculation with streptococci taken from the thoracic organs in doses several times more than sufficient to prove fatal for non-immunised animals.

Further, an animal immunised against the organism present in pleurisy and pneumonia resists doses 'several times more than fatal' from the strangles abscesses.

They hold that if we can protect animals against this streptococcus we shall prevent not only the usual symptoms of a benign attack of strangles, but also those too frequent and serious affections of the chest due to the same cause.

Antistreptococcic serum is obtained either from animals that have been already accidentally infected with such diseases, those that have recovered from strangles, or those that have been subjected to a series of inoculations with organisms (streptococci) of known strength.

Streptococci form but little toxin in culture media; immunisation is therefore carried out by means of the organism, entire cultures being injected, and the serum obtained is consequently antimicrobic.

Streptococci may be cultivated in peptonised bouillon and in some cases glucose bouillon, but they soon lose their activity in these media. If, however, horse or rabbit serum be added to the bouillon in the proportion of two of serum to one of broth it will preserve their activity.

Marmorek has shown that streptococci retain their virulence for more than a year in ascitic bouillon—*i.e.*, the fluid of ascites with one-third its volume of bouillon added.

In the preparation of anti-streptococcic serum the whole culture is injected in doses of from 3 to 5 c.c. After the

febrile reaction has subsided another dose is given, gradually increasing the dose until 500 or 600 c.c. of culture may be administered.

For the earlier injections the cultures are first sterilised by heating to 60° C. for three hours; later, when some degree of resistance has been acquired, living cultures are employed, and to prevent the formation of abscesses they are injected intravenously, commencing with small doses and gradually increasing the amount. The treatment lasts over twelve months.

The **serum is standarised**, according to Hewlett, by injecting a measured volume of serum subcutaneously into a rabbit, and at the same time ten minimum lethal doses of streptococci intravenously. Not more than 0·05 c.c. of serum should be required to preserve the life of the rabbit.

Marmorek's monovalent serum (see p. 46) was employed by Lignières in anasarca (purpura) with a certain amount of success, but it had no action against strangles.

The **polyvalent serum** (see p. 46) has been used with good results in cases of strangles, in 10-c.c. doses, repeated twice or thrice daily, and is reported by Professor Thomassen, of the Utrecht Veterinary School, to be spoken of highly both in Italy and Germany.

'The consensus of opinion in England appears to be that Marmorek's serum is of no avail in the treatment of equine strangles, and it is also useless in cases of purpura complicating that disease' (Proceedings of the National Veterinary Association of England, 1905). But it must be admitted that as yet we have hardly given the treatment a fair trial in this country. Theoretically, at any rate, the *polyvalent* serum should prove of service in the treatment of the numerous streptococcic diseases occurring in our patients.

In the case of purpura, Drouin (Alfort) observes that whilst under the old methods of treatment the mortality was

70 to 80 per cent., it fell to 19 per cent., and even so low as 15 per cent., when antistreptococcic serum (polyvalent) was employed. He recommends that 40 c.c. of serum be injected on the first day of treatment, and 20 c.c. on the following days. It is essential to commence treatment *early* in the course of the disease.

Dassonville and **De Wissocq** have also obtained a bacteriolytic anti-strangles serum from immunised horses (the mode of preparation corresponding to that already described), which acted—

1. As a *curative*, if used in sufficiently large doses (20 to 30 c.c.) at the commencement of the infection (strangles).

2. As a *preventive*. Animals already injected with the serum, and whilst still under its influence, resist inoculation with virus sufficient to produce considerable disorder in other horses. Young horses, after receiving 30 c.c. of this serum, may afterwards be injected with 2 c.c. of a virulent culture with no ill-effects, whereas the same culture injected into unvaccinated horses gave rise to widespread œdema and voluminous abscesses.

Anthrax.

Very few animals are possessed of a natural immunity against this disease; the domesticated herbivora, omnivora, and carnivora are readily affected, and unfortunately the disease is transmissible to man. Algerian sheep and white rats are refractory, but the last mentioned, under certain circumstances, may be successfully inoculated.

Anthrax is a true septicæmia, the *B. anthracis* being present in the blood of animals recently dead from the disease. This organism is large, rod-shaped, with square-cut ends, aerobic, non-motile, and between the temperature limits of 16° and 42° C., and in the presence of free

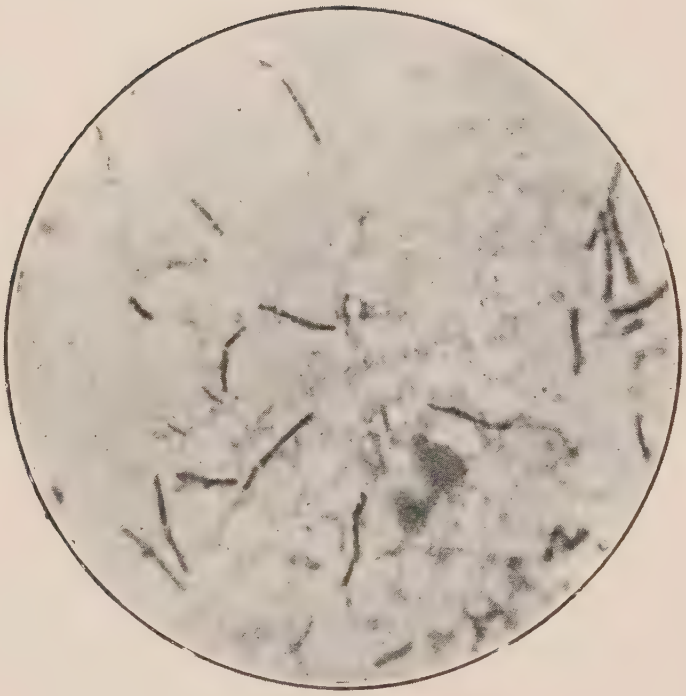


FIG. 24.—ANTHRAX BACILLI IN BLOOD OF GUINEA-PIG (× 1,000).

oxygen, forms in its interior a very highly resistant, centrally-placed spore. Spores are never formed in the living animal, and only in the dead when the carcass has been opened and the blood and organs containing bacilli have been exposed to the air at a suitable temperature.

Protective inoculation against anthrax is practised usually by inoculating susceptible animals with an attenuated virus (vaccine), although serum methods have been recently introduced, and will be referred to shortly.

When it is desired to attenuate cultures of the *B. anthracis* it is necessary to commence by preventing the formation of spores. This has been attempted by different workers in a variety of ways :

1. Chamberland and Roux cultivated the bacillus in weak antiseptic media—*e.g.*, carbolic acid bouillon (1 : 1,200 to 1 : 1,600), bichromate of potash (1 : 2,000 to 1 : 5,000), sulphuric acid (1 : 50), etc. It is said the power of forming spores may also be lost when the bacilli are cultivated for many generations on gelatin media.

Other methods of attenuating the virus are—

2. By heat (Toussaint, Pasteur, Chauveau).

3. Compressed oxygen (Chauveau).

4. Cultivating the organism in blood or serum of immune animals (white rats, frogs, etc.).

But the method of attenuating the virus most commonly followed is by means of heat.

About twenty-six years ago Toussaint inoculated sheep successfully with anthrax blood previously heated to a temperature of from 50° to 55° C. for ten to fifteen minutes. He erroneously considered that the bacilli were destroyed in this heating process. The later researches of Pasteur and Chauveau show that anthrax bacilli are not killed at a temperature of 55° C., but they are quickly attenuated in their virulence, and constitute, in fact, a virus-vaccine.

This method is still practised. Fresh non-sporulating cultures on agar or bouillon are heated to 50° to 55° C., and rabbits which have twice received 3 c.c., diluted, and inoculated intravenously, resist seven days afterwards the injection of virus fatal for control subjects.

Of the different methods of protecting animals against anthrax by inoculating them with an attenuated virus, that of **Pasteur** has been extensively adopted.

This scientist found that by cultivating anthrax bacilli at a temperature of from 42° to 43° C. in the presence of oxygen they cease to form spores, although still multiplying by fission (division). They undergo an *attenuation* proportionate to the period of incubation at this temperature, and at the end of six weeks their power of vegetation is entirely extinguished. The cultures lose their virulence by *degrees*, so it is possible to obtain a series of cultures of *varying degrees* of virulence.

For the purpose of immunising animals by Pasteur's method two vaccines of different degrees of virulence are employed :

The **first vaccine** consists of a bouillon culture of non-sporulating anthrax bacilli attenuated by cultivation for twenty-four days at a temperature of 42·5° C. *This vaccine should be capable of killing mice, but not guinea-pigs.*

The **second vaccine** is similarly prepared but has undergone the attenuation process only to the extent of twelve to fourteen days' incubation at 42·5° C. *This vaccine kills both mice and guinea-pigs, but not rabbits.*

Animals are first inoculated with the more attenuated or first vaccine, and after an interval of from ten to fourteen days with the stronger, less attenuated second vaccine.

Ten days to one month after the second inoculation the immunity is established. This lasts about one year ; consequently, the operation should be repeated annually.

The **dose** of each vaccine for horses, mules, and cattle s $\frac{1}{4}$ c.c., and for sheep and goats $\frac{1}{8}$ c.c. They are injected subcutaneously behind the shoulder in the ox, inside the thigh of the sheep, and in horses and mules in the neck region, to prevent chafing of the inoculation area by the collar or harness.

It is customary to inoculate the first vaccine on one side of the animal, the second on the other.

Accidents occasionally follow the inoculation of Pasteur's vaccine, the animals contracting the disease in a fatal form as a result of the injection. This is especially the case in sheep; these animals are *naturally* very susceptible to anthrax. True, the losses attributable to vaccination are not high—about $\frac{1}{2}$ per cent., calculated on a very large number of vaccinations—still, as pointed out by McFadyean, they are not equally distributed, and the losses of individual owners may be as high as 10 per cent., whilst inoculations on other farms may result in no deaths whatever. Apparently the strength of Pasteur's vaccines cannot be regulated with absolute certainty; nevertheless, they have rendered good service in protecting animals against this rapidly fatal malady.

According to Koch, whilst Pasteur's vaccines render sheep and cattle immune to inoculated anthrax, they do not protect against natural infection by way of the intestinal tract.

Anti-anthrax Serum.

By injecting animals already immunised by means of Pasteur's vaccines, with large quantities of virulent culture —hyper-immunising them, in fact—a bacteriolytic serum is prepared, and this has been successfully used as a curative agent for local anthrax (malignant pustule) in man.

Sclavo found that—

1. Sheep brought to a high degree of immunity furnish a serum active against anthrax.

2. This serum showed prophylactic and curative power against anthrax when inoculated into rabbits.

Later he found it possible to obtain a more powerful serum from the ass, and now prepares his serum by hyper-immunising that animal.

After having undergone the process of immunisation, blood is withdrawn from the jugular, the serum decanted, and ether added to the extent of 3 per cent. of the whole bulk.

To test the value of the serum six rabbits are used (three acting as controls). These six animals are inoculated sub-cutaneously in the abdominal region with 0·5 c.c. of a suspension of an anthrax culture. Three of the rabbits receive at the same time 10 c.c. of the serum injected into one of the auricular veins.

The control animals not having received the serum die in about thirty-six hours, whilst the three animals which received serum at the same time as culture remain well.

The efficacy of the serum having thus been proved, it is introduced under aseptic precautions into 10-c.c. tubes, from which it is used.

As pointed out by Stockman, in the lower animals little can be expected from anti-anthrax serum as a *curative* agent, as one seldom suspects anthrax in these subjects until they are found in a dying condition.

Sobernheim has utilised an anti-anthrax serum for the purpose of protecting animals. He uses serum and a culture of about the strength of Pasteur's second vaccine injected at the same time, but in different parts of the body. Only one inoculation is required, and in dealing with large herds of cattle this alone is a favourable consideration.

'To test the efficacy of this combined method Sobern-

heim experimented on thirty-three sheep, eighteen oxen, and some horses. From 5 to 15 c.c. of serum were inoculated into the left side of the animals, and immediately afterwards 0·5 to 1 c.c. of slightly attenuated anthrax culture was injected into the right side. After twelve to fourteen days inoculations with virulent cultures were made.

'The results were entirely satisfactory. Ten c.c. of serum sufficed to protect, whilst all the control animals which received the virus unaccompanied by serum died in twenty-four to thirty-six hours.

'Further, the immunity so conferred protected three out of four sheep from contracting anthrax by way of the *intestinal* tract.

'Its *curative* power was also shown in that of five sheep inoculated with virulent anthrax and then subcutaneously injected at intervals of from ten minutes to six hours later with 40 c.c. of serum; two animals survived, whilst death was delayed in the others from four to eight days.'

'Sobernheim summarises the advantages of this combined active and passive immunising method over that of Pasteur as follows:

'1. It is free from danger.

'2. It is effective in one day, and has not to be repeated.

'3. A stronger dose and more active cultures are used than is the case with Pasteur's vaccine, and therefore probably a more lasting immunity is conferred.

'4. It protects from infection by way of the intestinal tract.

'5. It can be used for curative purposes' (Legge, Milroy Lectures, 1905).

Carini believes that, in spite of the rapid and almost sudden course of infection in animals, an anti-anthrax serum may be used with good results.

He states:

1. Anti-anthrax serotherapy may be used in practice, and

should be suitable treatment in every case taken at the commencement; it must be used in large and repeated doses (150 to 200 c.c. in all).

2. Ten cubic centimetres of serum are not sufficient to insure immunity in cows against anthrax; it would be necessary to choose 20 c.c. at least as an immunising dose.

3. As soon as anthrax is noticed in a shed the temperature of all the cows should be taken at least twice every day, until immunisation of the whole of the herd has been obtained. The surest means of obtaining this is to make a provisional immunisation with 20 c.c. of serum, to be followed by active immunisation by means of the Pasteurian vaccines.

Tetanus.

In this disease the causal organism, the tetanus bacillus, remains strictly localised to the infected wound; here it manufactures a poison (tetanine or tetano-toxin); this, after absorption, travels to the central nervous system, with the cells of which it enters into combination, thereby damaging them and giving rise to the characteristic and well-known symptoms of tetanus.

The tetanus bacillus is a motile rod-shaped organism, and grows only in the absence of oxygen (anaerobic). When sporulating, it presents the appearance of a drum-stick or pin, the head being formed by the spore; this is terminal, and of a diameter greater than that of the bacillus.

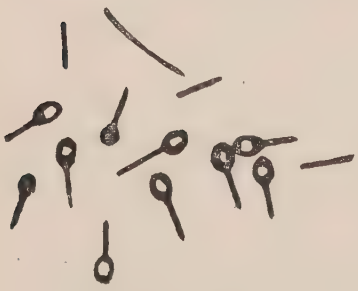


FIG. 25.—B. TETANI.

The bacilli or their spores are widely distributed in nature; they are found in dust, soil, water, manure, and in the alimentary canal of healthy horses.

Only when implanted in a wound can the micro-organism give rise to tetanus. It can flourish best in deep, punctured, lacerated, and contused wounds.

From the wound the tetanus toxin travels along the peripheral nerve trunks to the central nervous system. Large doses may in part be absorbed by the circulatory system, but, according to Meyer and Ransom, even in this event the poison is taken up from the blood by the nerve endings, and transmitted along the axis cylinders of the nerves to the brain and cord.

The toxin has a peculiar affinity for the nervous structures, and readily enters into combination with cerebral cells, either in the animal body or when experimentally mixed with them *in vitro*.

Wassermann has shown that if tetanus toxin be mixed with brain matter pounded in a mortar the toxin is fixed by the nerve cells, and neutralised or rendered inert; so much is this the case that a fatal dose treated in this way is no longer able to produce fatal results when injected into a susceptible animal;—one line of treatment is based on this fact, and will be referred to shortly.

Animals inoculated with small and gradually increasing doses of filtered tetanus toxin acquire an active immunity against the poison. The blood-serum of animals so immunised is possessed of antitoxic properties, and will, when injected into susceptible animals, confer on them a temporary (passive) immunity against tetanus.

Preparation of Tetanus Antitoxin.

1. First, it is necessary to obtain pure cultures of the tetanus bacilli; these are subinoculated into bouillon in special flasks, and cultivated in an atmosphere of hydrogen for about four weeks, until extremely toxic.

2. The culture is then filtered through unglazed porcelain, to obtain the toxin free from bacilli.

3. A young horse is now selected, one previously tested with mallein and tuberculin to insure its freedom from glanders and tuberculosis.

4. The treatment of such an animal for the production of antitoxic serum is commenced either with extremely minute doses of tetano-toxin (fractions of a minim), or with a toxin attenuated by heat, or by adding to it antitoxin, or chemical agents such as a solution of iodine. Afterwards, as the animal acquires some tolerance, pure toxin may be administered.

The injections are given at intervals of a few days, gradually increasing the doses of toxin until, after some months, ounces may be administered, and at the end of twelve or eighteen months' treatment a pint or more will produce no ill-effect.

At frequent intervals during the process of immunisation the horse is bled, and its serum tested experimentally as to its power to protect mice and guinea-pigs against tetanus.

5. When the serum has attained a sufficient value, eight to fourteen days after the last injection blood is drawn from the jugular vein by means of a sterile cannula into sterilised glass vessels, and allowed to coagulate on ice.

After about twenty-four hours the serum is drawn off by means of sterile glass pipettes, filtered, and bottled aseptically. Occasionally 0·2 to 0·5 per cent. of carbolic acid is added as a preservative.

Stored in a dark, cool place, the serum will keep unaltered for a considerable length of time.

It is used either in the liquid state, or desiccated (dried *in vacuo* over sulphuric acid and subsequently pulverised). This dried preparation will retain its activity for years, and

is extremely useful on that account. When required for use, the powder can be dissolved in water which has been boiled and allowed to cool again. One gramme of the dry substance corresponds to about 10 c.c. of fluid serum.

Doses.—'An efficient serum acts as a **preventive** in doses of 10 to 20 c.c. for the larger, and 5 to 10 c.c. for the smaller, animals.'

'As a **curative** much larger doses have been used—50 to 100 c.c. Babes recommends a dose for man of 300 to 500 c.c., and for the horse 8 to 10 *ounces*' (Law).

In veterinary practice antitetanic serum is used to protect animals from tetanus, being injected into them when for any reason they are especially liable to contract the disease (such as the presence of wounds, prior to operations, etc.).

The immunity given to an animal by injections of the serum is soon lost; the immunity derived from one dose lasts about three weeks, sometimes less, so that to immunise an animal for a period long enough to allow the majority of wounds to heal it would be necessary to give two injections at intervals of ten days.

As a preventive of tetanus, used in serious operations or wounds likely to be followed by tetanus, the serum has proved efficacious, but for the treatment of cases of tetanus actually developed it is now generally admitted to be of little practical value.

When the toxin has *firmly* combined with the cells of the nervous system, *as manifested by the appearance of symptoms of tetanus*, no amount of antitoxic serum can withdraw the poison or counteract its effects, although it will, of course, neutralise any *excess* of toxin which may be circulating in the blood-stream.

Nocard demonstrated that 'even 10 grammes of dried antitoxin had no protective action against a minimum fatal dose of toxin unless injected forty-eight hours before the

appearance of the first symptoms—that is to say, three to four days after the toxin, which in the horse takes on an average five days to act.'

Calmette has used the **dried** antitetanic **serum as a dressing for wounds** likely to be followed by tetanus. He finds that if the wound is only dressed with liquid serum immunity is doubtful, but if a small quantity of dry serum finely powdered is dusted on it is certain, and a few milligrammes render animals resistant to a toxic dose multiplied by ten.' This method is only a preventive, not curative, and appears to offer no advantages over the subcutaneous or intravenous injection of antitetanic serum.

Fiebiger, working on the principle that tetanus-toxin combines with nervous matter, used an **emulsion of brain** injected into affected animals as a therapeutic measure, the object being to effect a combination with the toxin before it (the toxin) can reach the nerve centres. The brain emulsion acts in this respect exactly in the same way as the antitetanic serum. Fiebiger first used the brain of a rabbit, but later utilised lambs' brains. The brain of a recently slaughtered lamb is rubbed down with physiological salt solution (see Appendix) in a glass vessel, using a glass pestle and observing aseptic precautions. About $\frac{1}{2}$ litre of emulsion is injected under the skin or panniculus. It is stated that half the cases of tetanus recovered when treated in this way; the majority received only one injection, but some two or three.

The method of injecting cerebral emulsion offers no special advantages over the use of antitetanic serum, and it would seem that the latter should always be preferred, if only for the fact that it is more readily absorbed. Nevertheless, if for any reason a supply of antitetanic serum is unobtainable, then brain emulsion might be advantageously employed.

Roux and Borrel advised injecting the antitoxin under

the dura mater as a therapeutic measure. This method is based on the fact that the lower and less important centres are first affected by the toxin, and one may, by **subdural,** or by the **intracerebral injection of antitoxin,** get in advance of the toxin and immunise the higher centres before the latter can reach them. Sendrail and Cuillé (*Revue Vétérinaire*) found that unfavourable results attended this operation—*i.e.*, subdural injection—and in their hands the treatment aggravated the symptoms in tetanised horses and mules. Stockman reports that he tried the method unsuccessfully on two cases (horses), and both died. Certain observers have reported successful results, but, as the last-mentioned writer observes, 'it must not be forgotten that a large number of successes are necessary with a method of this kind before one can eliminate from one's mind the possibility that the patient would have recovered had the treatment been withheld.'

Standardisation of Tetanus Antitoxin.

According to Hewlett, 'the original method of standardising tetanus antitoxin was by finding the minimum amount of the antitoxin which, when injected simultaneously with the minimal lethal dose of tetanus toxin, completely protected a guinea-pig of 500 grammes weight. The weight of guinea-pig in grammes which is so protected by 1 c.c. of the serum gives the value of the serum in units. Thus, if from the tests 1,000 guinea-pigs, each weighing 500 grammes, were protected by 1 c.c., then the value of the serum would be said to be 500,000 (500 × 1,000). But this method has many objections; in using a single lethal dose of toxin it is evident that if only a small fraction of the toxin be neutralised death will not ensue, and therefore the method may fail to give the actual immunising value of the antitoxin.

'It is therefore preferable and customary where the above (Roux's) method is employed to work with a multiple of the minimal lethal dose, generally ten lethal doses, so that a unit would be that amount of serum which would completely protect a 500-gramme guinea-pig from ten minimal lethal doses.

'No tetanus antitoxic serum for therapeutic use should possess a less potency than 1,000,000, as reckoned by the Roux unit—*i.e.*, 1 c.c. should be sufficient to protect 1,000,000 grammes of guinea-pig against the minimal lethal dose of tetanus toxin.' Behring has recently devised a more delicate method of standardisation. For further details the reader should consult one of the larger works.

Diphtheria Antitoxin.

Diphtheria in man is due to the presence locally in the diphtheritic membranes in the throat and upper air-passages of the Klebs-Löffler bacillus; here it manufactures a toxin, and this after absorption gives rise to serious general phenomena.

Under natural conditions infection by means of the Klebs-Loeffler bacillus (*B. diphtheriæ*) does not occur in the lower animals. Certainly we have a diphtheria in calves, and also avian diphtheria, but these diseases have no connection with the diphtheria occurring in man, and are caused by totally different micro-organisms.

Serum-therapy in the case of human diphtheria, then, is only of secondary interest to veterinarians. It is sufficient to observe that a valuable diphtheria antitoxic serum is prepared in a manner similar to that for antitetanic serum, by treating horses with gradually increasing doses of filtered diphtheria toxin. After a time this animal furnishes a prophylactic and curative antidiphtheria serum, which has been used with most successful results.

Black Quarter

(*Quarter Ill, Quarter Evil, Black Leg*).

This disease of young cattle, goats, and sheep is characterised by the presence in animals affected of a specific lesion in some part of the body (usually the limbs) and in connection with the muscular tissue. This appears at first as a firm, painful inflammatory engorgement, which rapidly extends in area and depth, finally becoming insensitive, emphysematous, and resonant.

Associated with the presence of this characteristic swelling are the usual signs of fever, more or less intense, and the patients generally succumb in the course of thirty-six to forty-eight hours.

The causal agent is a micro-organism, the *B. sarcophysematos bovis*, or *Bacillus Chauvæi*, present in abundance in the local lesion, but not in the blood or lymph streams during life.

This organism is motile, anaerobic, rod-shaped (with rounded extremities), and when sporulating appears 'bell-clapper' or club-shaped, the spore being terminal.

FIG. 26.—B. SARCOPHYSEMATOS BOVIS.

Immunisation.—One attack of black quarter confers on the subject insusceptibility against a second. A variety of methods have been introduced and practised at different times to bring about this result artificially.

1. The first method, and one which in practice has yielded good results, is that introduced originally by Arloing, Cornevin, and Thomas. This consists in the employment of two vaccines of different degrees of virulence,

prepared from the natural virus attenuated by exposure to high temperatures.

The mode of preparation of these vaccines is as follows: In order to obtain the natural virus free from contamination with organisms other than the black quarter bacillus, a calf is inoculated with the virus of black quarter, and immediately after the death of the animal strips of muscular tissue are dissected out from the characteristic lesion and dried in an oven at a temperature of 32° to 37° C. When quite dry, the muscle—which is, of course, extremely virulent—is powdered, and mixed with water to form a thick paste. This paste is then spread on glass plates, and these are placed in a thermostat and left there for six or seven hours.

To prepare the **first vaccine** the thermostat is maintained at a temperature of 103° C., whilst for the **second** it is kept at about 93° C. (between 90° and 95° C.).

When the plates are taken out of the thermostat it is found that the paste has dried in the form of a brown crust; this is removed and carefully ground in a sterilised mortar to form a fine powder. These dried vaccines are then introduced into small sterilised glass vials, 0·1 gramme of either the first or the second vaccine being placed in each tube.

The dose of either vaccine is 1 centigramme; consequently, a vial such as the one here mentioned would contain ten doses.

The dried vaccines, if stored in a dry, cool, and dark place, will retain their activity unimpaired for a considerable length of time. When required for use, the powder may be rubbed down with a measured quantity of sterile water in a sterilised mortar. For instance, the contents of one vial (0·1 gramme) might be mixed with 5 c.c. of water; of this, 0·5 c.c. would, of course, constitute one dose of either the first or second vaccine, as the case may be. The vaccines are inoculated subcutaneously, the site usually selected being the tail, near

its extremity; the first, or weaker, vaccine is first introduced, and in the course of from seven to ten days this is followed by the second stronger one.

Instead of introducing the vaccines by means of a hypodermic syringe, as above indicated, the dry vaccine may be mixed with water to form a thick paste, and twisted cotton threads soaked therein, and these can be inserted as setons under the skin. Commercially this 'cord form of vaccines' has been issued by the Pasteur Vaccine Company under the name of 'Blacklegine.' Each cord holds one dose of either the first or the second vaccine.

Immunity against black quarter is acquired by the animal about one week after inoculation with the second vaccine, and lasts, at any rate, for a year.

2. **Immunisation by Means of a Single Vaccine.**—Kitt's single vaccine is prepared by subjecting the virus (either the natural virus or a culture of the black quarter bacillus) to attenuation by moist heat at 98° to 100° C. for six hours. This vaccine is subsequently dried and used in decigramme doses, inoculated subcutaneously in the shoulder region, in a manner similar to that already described.

The advantage of this method lies in the fact that only one vaccine is employed, and the whole process of immunisation is completed at one operation.

On the other hand, the double method—the employment of two vaccines of different degrees of virulence—appears to be safer and to confer a more lasting immunity. Therefore, when circumstances permit, one should give preference to the method of Arloing and Thomas.

3. Immunisation by the intravenous injection of a small quantity of virus has also been practised, and results in immunity, but extreme care is required in performing the operation; should the least portion of virus come in contact with the subcutaneous cellular tissue a fatal issue may ensue.

4. The above-mentioned authority (Kitt) prepared a protective **serum** by injecting a cow with natural virus, at first intravenously, afterwards subcutaneously, until it ceased to react. He found that 10 c.c. of a serum so prepared sufficed to protect sheep against a fatal dose of strong virus; but the results were somewhat uncertain.

Leclainche and Vallée attempted the immunisation of cattle against black quarter by inoculating them subcutaneously with from 2 to 3 c.c. of culture previously heated to 70°, 65°, or even 60° C. In the first series of experiments animals so inoculated when tested nine days afterwards successfully resisted the intramuscular injection of virulent juice which killed control subjects in thirty hours. But in subsequent experiments to test the value of this method of immunisation some of the subjects died from black quarter attributable to the inoculation.

These two authors next turned their attention to the preparation of a serum by inoculating horses intravenously with virulent culture of the black quarter bacillus. But experiments conducted to ascertain the value of vaccination by the *combined* employment of such a serum and virus (*i.e.*, a mixture) gave uncertain results.

Finally, they introduced the method of vaccination against black quarter by the **successive inoculation of serum and virus**.

They say (*Annales de l'Institut Pasteur*): 'As a rule, when an ox receives under its skin 10 c.c. of serum, and then twenty-four hours afterwards 10 drops of virulent serosity, it presents an intense thermal reaction (more than 2°), which lasts for two or three days. The local reaction is manifested by a flat œdematous swelling as large as the hand.

'The animals which react in this way acquire a solid and durable immunity. Subsequently they resist inoculation with 1 c.c. of virulent serosity into the muscles.

'Our experiments on vaccination have been carried out in the following fashion: the animals (young cattle) receive an injection of from 10 to 20 c.c. of serum, according to their weight. Then five to eight days afterwards they are inoculated subcutaneously at the shoulder, flank, or tail with 1 c.c. of pure culture which has been heated for three hours at 70° C.'

For the protection of valuable young cattle the successive employment of serum and virus is decidedly the safest method. For less valuable stock, where a simpler procedure may be desired, then the inoculation with vaccine virus alone may be preferable, but is occasionally attended with fatal results.

Regarding the best age and time to vaccinate, as already observed, the disease is especially common in young cattle. Calves whilst on a purely milk diet are seldom attacked, whilst cattle over four years old are no longer susceptible.[1] Occasionally calves of even one week old have suffered from the disease; consequently, one may vaccinate at that age. But as a rule, vaccination may be deferred until the animals are from three to six months old.

In districts in which the disease is prevalent and where the attenuated virus (virus-vaccine) alone is employed, it is advisable to revaccinate each year during the first two or three years of the young animal's life.

The best time of the year to vaccinate is *early* in the spring or autumn, as during those seasons the disease usually makes its appearance.

[1] This statement regarding the insusceptibility of older cattle applies only to those indigenous to a black quarter infected district. Probably such animals, prior to attaining the age of four years, have suffered from a mild and unrecognised attack of black quarter naturally contracted, and so acquired immunity.

Louping Ill and Braxy.

The two ovine diseases louping ill and braxy have recently formed the subject of investigation by a Committee appointed by the Board of Agriculture. An exhaustive report has been issued, and from this the following short notes have been extracted.

Louping ill (*Chorea Paralytica Ovis*) prevails amongst sheep during the *spring* months in the western and southern districts of Scotland, and in England north of the Tyne. So far the disease has not made its appearance outside Great Britain.

Sheep of all ages are subject to the malady, but it occurs with great frequency amongst yearlings.

The symptoms commence with dulness and reeling gait, and are succeeded by 'choreic spasms and tetanus-like rigidity,' terminating in more or less complete paralysis of the extremities.

Braxy (*Morbus Subitarius Ovis*) prevails over a large part of Scotland, the North of England ; also in Cornwall, Gloucestershire, Wiltshire, and a few other counties; Wales, and along the west coast of Ireland ; also in Norway, Iceland, and the Faroe Isles, making its appearance, in the United Kingdom at any rate, only *during the late autumn and early winter months.*

The symptoms appear suddenly, and consist chiefly of dulness, followed in a few hours by complete collapse and death.

The Committee consider that 'the organisms respectively of louping ill, braxy, black quarter, malignant œdema, the disease of sheep known as "struck," and probably several others, belong to a group the individuals of which evidently possess a very close mutual relationship. They are all rod-shaped and sporing anaerobes, whose natural habitat is the

intestinal canal. It is only occasionally they pass the intestinal wall and begin to germinate upon the liquids and tissues of the body, and under these circumstances the peritoneal cavity is the part first invaded. They all grow luxuriantly upon its secretion, and may be obtained from it in a state of almost pure culture.'

So that the organisms of both louping ill and braxy belong to a group of which the black quarter bacillus may be taken as a representative. To this organism they apparently bear a close resemblance in shape, method of cultivation, sporulation, etc.

The **louping ill bacillus** is not found in the blood, tissues, or cerebro-spinal fluid. Its toxins are *intracellular* (confined to the interior of the bacterial bodies). No extracellular toxins are formed, as, for example, is the case with the *B. tetani*.

Now, as no extracellular toxins are formed, and in addition the organisms are absent from the cerebro-spinal fluid and the blood of affected animals, it would appear difficult at first sight to trace the relationship between cause and effect in this instance. The Departmental Committee, however, have advanced a theory explaining not only the manner in which the symptoms are produced, but accounting also for the seasonal occurrence of this and certain other diseases.

During certain seasons of the year (summer, autumn, and winter), they say, sheep's blood is strongly bactericidal for the louping ill bacillus; if blood be drawn from a sheep during these seasons, mixed with the microbes, and subsequently incubated, no growth takes place—quite the reverse, for the organisms are promptly dissolved and destroyed.

But during the **spring** months (the louping ill season) sheep's blood becomes less bactericidal for the bacillus of

louping ill—in fact, at this season it forms a suitable culture medium for that organism.

The **same applies to braxy.** During the **autumn and early winter** months (the braxy season) sheep's blood loses its bactericidal effect for the braxy bacillus, whilst at other seasons it is possessed of strong bacteriolytic action against that microbe.

In louping ill districts the specific germ, ever present, is constantly gaining access to the animal system by means of forage, etc., yet, in consequence of the strong bactericidal power of the blood during the greater part of the year, it is unable to do harm ; the intestinal walls form an effectual barrier, and the bacilli pass along the intestinal tract without giving rise to recognisable symptoms.

Not so, however, during the spring months. Now the blood is less bactericidal for the organism, and it can pass through the intestinal wall, overcoming the resistance (now enfeebled) of the blood contained therein. Still, the bacilli gaining the blood-stream are bacteriolysed sooner or later, and in this way their toxims (intracellular) are set free, and these freed toxins act on the central nervous system, giving rise to the characteristic symptoms of louping ill. In time the invading bacilli are reinforced, and ultimately gain the peritoneal fluid ; after this death is not long delayed.

The **braxy** bacillus, like the louping ill, and black quarter organisms, gains access to the animal host by way of the alimentary tract, and it is found in the peritoneal fluid, intestinal contents, and *the blood* of affected animals.

'The cause of the seasonal character of the disease is evidently the same as in louping ill—namely, that during certain months of the year—those in which the disease prevails (autumn and early winter)—the blood constitutes an excellent medium of culture for the bacillus, while during the remainder of the year it is bactericidal to it.'

Immunisation against Louping Ill.

The Committee are of opinion that 'a natural process of immunisation goes on in "seasoned" sheep, and those which die in the spring have not acquired this. The means by which the former have become immune is that they have taken the organism into the alimentary tract at a time when they are insusceptible to the disease. The clear indications for preventive treatment, therefore, are to imitate nature as far as possible — to administer the organism at a time when it proves harmless.'

This object is attained by administering *per os* to susceptible sheep during the month of January—*i.e.*, prior to the louping ill season—glucose bouillon cultures of the louping ill bacillus. Two doses are given, with an interval of seven to ten days between them. The first dose consists of 4 c.c. of a thirty-six hours culture of the louping ill bacillus—that is, cultivated in an incubator for thirty-six hours. The second is formed of a like quantity of a culture aged but twenty-four hours.

In each case, before dosing the sheep, the 4 c.c. of culture are diluted by the addition of 50 c.c. of water.

It is stated that this method is free from danger, and has proved completely successful.

Experiments were conducted to test the value of subcutaneously injecting the cultures, but this proved an extremely dangerous procedure, giving rise to widespread œdema and grave constitutional disturbance.

Immunisation against Braxy.

The method of immunising animals against braxy corresponds largely to that for louping ill—namely, in administering cultures of the specific organism by way of the alimentary

tract at a season of the year when the subject is insusceptible to its action.

The prophylactic consists of glucose bouillon cultures of the braxy bacillus. Two doses are given, with an interval of ten to fourteen days between them.

At the first dosing, 4 c.c. of a thirty-six hours' culture are given, and at the second a like quantity of one twenty-four hours old. Each dose is diluted with 50 c.c. of water before being administered to the sheep.

It is advised that the process be carried out during the month of August—that is, before the commencement of the braxy season.

This method, affirm the Committee, is free from danger, and so effectual that any mortality is practically hardly worth considering.

The subcutaneous inoculation of the bacillus proved dangerous, and this method is impracticable.

Other Diseases resembling Braxy.

The Departmental Committee arrived at the conclusion that at least three other diseases occur amongst sheep during the braxy season, and these have all been confounded with braxy.

These three diseases—one being malignant œdema, and the other two as yet unnamed—are apparently caused by organisms closely resembling the braxy bacillus, but differing therefrom in certain features.

Administration of the braxy bacillus immunises only against braxy, and not against these other closely-related diseases ; but it is believed that the latter may be treated on the same general principles, and perhaps it may eventually be found possible to immunise an animal against several or all of these diseases at one and the same time, by administering

to it at non-susceptible seasons a **mixture** of cultures of the various causal microbes.

Further, it appears possible to immunise against louping ill and braxy at one operation, for during the Committee's investigations cultures of *both* braxy and louping ill bacilli were given to twenty-five sheep in the early autumn.

The first dose, consisting of 4 c.c. of *each* culture, was administered on September 13, and the second, another 8 c.c.—*i.e.*, 4 c.c. of *each* organism—on September 30.

Although these animals were pastured on land which 'claimed a high mortality from both diseases, not a single death occurred amongst them.'

In the last few pages of this highly interesting report the following remark occurs: 'This subject of *multiple immunisation* through the intestine opens up a field of research fraught with the most profound practical issues. Indeed, the whole subject of immunising through the intestine is one which has bearings of a far-reaching character relative not only to the diseases of the lower animals, but also to those which are peculiar to man.'

Tuberculosis.

Tuberculosis, a disease caused by the well-known tubercle bacillus of Koch, affects mankind and all the domesticated animals—in order of susceptibility cattle, swine, horses, dogs, cats, sheep, and goats. Birds are frequent victims to the malady, and it is not unknown amongst the cold-blooded animals.

The tubercle bacillus is a straight or slightly bent, small, thin, non-motile, rod-shaped organism with rounded ends (see Figs. 3 and 4). It is stained with difficulty, but once stained, retains the colour with tenacity, resisting decolorisa-

tion even with the strong acid solutions; it belongs, in fact, to the 'acid-fast' group of organisms.

It is a purely parasitic organism, and therefore not easily cultivated on artificial media; on these it grows very slowly, under aerobic conditions only, and when maintained at a temperature corresponding to that of the animal from which it may have been derived.

'It has been said that the continued cultivation of the tubercle bacillus upon culture media lessens its parasitic nature, and prolonged cultivation destroys its virulence' (McFarland).

Koch, at the International Congress, London, 1901, startled the world by his statement that tuberculosis of cattle and man owe their origin to different microbes, and that transmission from one species to the other does not occur.

The tubercle bacilli from human and bovine sources are not identical; the injection of 5 centigrammes of *human* bacilli under the skin of cattle produces only a local reaction at the site of inoculation, the infection spreading only as far as the nearest lymph gland.

On the other hand, the injection into cattle of 5 centigrammes of *bovine* tubercle bacilli is followed by disastrous results, producing not only an intense local reaction, spreading to the nearest lymph glands, but also a generalised tuberculosis, and the inoculated animals often succumb in the course of six to eight weeks.

So that human bacilli are generally non-virulent for cattle; but whether bovine bacilli are harmless to man has not been determined, since it is, of course, impossible to inoculate the latter experimentally with bovine bacilli.

Kossel and Weber find that in a considerable number of cases tuberculous lesions in man produced by bovine bacilli remain limited to the point of entry of the organisms

and to the nearest lymph glands, or to these latter alone. *Nevertheless, they consider that in some cases the lesions may extend and prove fatal.*

Human and bovine tubercle bacilli present certain other distinguishing features; human bacilli are slightly thinner and longer, and may be cultivated somewhat more readily on artificial media.

Amongst the domesticated animals other than cattle—*i.e.*, horses, swine, dogs, etc.—it is probable that infection may occur from both human and bovine sources, but for these animals in every case bovine bacilli are the more virulent.

Tuberculosis in the fowl is produced by still another variety of the tubercle bacillus, one differing in many respects from the mammalian organism. The avian bacillus is longer, but otherwise presents the same shape and staining reaction and gives rise to the same products in its growth as does the mammalian bacillus, but it differs from the latter in growing much more readily on culture media and at a higher temperature (43° to 45° C.; the mammalian bacillus ceases to grow at 41° C.), and in contrast to the dry, scaly cultures of the mammalian organism, those from the fowl appear quite moist.

Tubercle bacilli from the fowl are not easily transmitted to mammals, whilst birds resist inoculation with mammalian tuberculous material. This statement does not apply to parrots, which, strange to say, appear to differ from most other birds in being susceptible to the mammalian tubercle bacilli.

Many authorities distinguish three types of tubercle bacilli:

1. The human type.
2. The bovine type.
3. The avian type.

Whether these three varieties—human, bovine, and avian —constitute three distinct species, or are merely different types of the one species, modified, it may be, in passing through the different hosts, still remains a debatable point.

On this matter Kossel, at the Paris Congress (1905), expressed himself as follows: 'When taking note of all these circumstances, one cannot avoid the conclusion that two types of mammalian tubercle bacilli exist, and that tuberculosis of cattle owes its distribution to bacilli of the one type (typus bovinus), whilst human tuberculosis is spread chiefly by another type (typus humanus). A third tubercle bacillus entirely differing from the two types described above is found in tuberculous fowls. This so-called avian tubercle bacillus is recognised as different even by some of those who do not agree with the separation into two types of the mammalian tubercle bacilli.'

Artificial Immunisation of Cattle against Tuberculosis.

So long ago as 1891 efforts were made to immunise animals against virulent tubercle bacilli by inoculating them with tubercle bacilli of low virulence.

McFadyean, in 1901 and 1902, reported that by inoculating cattle with tubercle cultures of low virulence their resistance to tuberculosis had been considerably increased. He states: 'It appears to be justifiable to conclude that whatever may have been the degree of natural immunity possessed by these three experimental animals, it was much increased by the successive intravenous inoculations to which they were subjected. The immunity was not absolute, but it may be doubted whether a degree of resistance that would merit that term is obtainable by any method in cattle.

Von Behring's method of immunising cattle against tuberculosis, first published in December, 1901, is based on the use of tubercle bacilli of human origin non-virulent for cattle.

He employs a standard culture obtained originally in 1895 by infecting a guinea pig with phthisical sputum, and since maintained in the laboratory.

The bacilli are dried *in vacuo*, and afterwards moistened with a few drops of glycerine and rubbed up in a short-necked Wurtz mortar by means of glass balls. They are then emulsified gradually in a given quantity of normal saline solution, with 1·5 parts of sodium carbonate to every 1,000 parts. The addition of this salt favours the homogeneity of the emulsion. Every 2 c.c. of the emulsion contains 4 milligrammes of dried bacilli.

For the purpose of immunising cattle against tuberculosis two inoculations are given intravenously, with an interval of about twelve weeks between them. The dose for the first inoculation is 4 milligrammes of dried bacilli, and for the second 20 milligrammes.

The resistance to tuberculosis of cattle treated by Von Behring's method is undoubtedly increased ; this has been proved by numerous experiments, notably those at Melun, where several young and healthy cattle were vaccinated, and afterwards divided into three lots :

1. Seven vaccinated and seven healthy unvaccinated cows of the same breed and age were all inoculated under the skin of the shoulder with tuberculous virus.

One month later all the control animals showed vast tuberculous lesions, while all the vaccinated beasts were free, with the exception of one or two which showed trifling traces of the affection.

2. Six vaccinated and six unvaccinated animals had virulent cultures injected into the jugular vein.

The six vaccinated animals remained in good health, whilst all the controls were gravely affected, three dying in a fortnight.

3. Vaccinated cows were placed alongside tuberculous animals in the same shed. The vaccinated animals resisted, whilst on post-mortem examination the controls showed generalised tuberculosis.

Certain workers have immunised animals by subcutaneous instead of intravenous injection; others employ in the process attenuated tubercle bacilli of other than human origin—equine, bovine, etc.; others, again, use human bacilli passed through cold-blooded animals. Roux, Vallée, and Carrée used as vaccine bacilli of equine origin; they consider this method superior to that of Von Behring, as by avoiding the use of human tubercle bacilli there is less danger to man when vaccinating cattle.

'Different types of tubercle bacilli have different values in the process of immunisation. 'Avian tubercle bacilli produce a progressive intoxication with profound emaciation; but avian cultures do not appear to confer upon animals so much immunity as results from the use of mammalian cultures that are non-virulent for the animal upon which they are used. So that to produce an artificial immunity in mammals it seems necessary to vaccinate with an attenuated culture of mammalian bacilli' (Pearson).

Koch states: 'The results that have been attained as regards preventive inoculations against tuberculosis in cattle are very varied, *but the most satisfactory have been from the intravenous injection of living human bacilli.*

'Immunity is conferred in about three months, and lasts for at least a year.'

On the subject of the protective inoculation of cattle against tuberculosis Huytra formulates the following conclusions:

1. An intravenous injection, once repeated, of human tubercle bacilli, after Von Behring's method, or some similar one, increases to a very considerable extent the power of resistance of cattle to artificial infection with bovine bacilli.

2. The question whether, and if so how far, the immunity produced in this way extends to natural infection finds no solution in the results of past experience. To solve this problem accurate observation of inoculated animals continued for years will still be necessary.

3. The process is innocuous to sound cattle, and presents no difficulties in carrying into practice.

This last conclusion is not wholly correct. It is true that the injection of living tubercle bacilli, non-virulent for cattle (*i.e.*, Von Behring's bovo-vaccine), is apparently harmless to young calves, but it is not without danger to adult animals.

Doses withstood by calves, when intravenously injected into older animals, have often provoked a fatal issue from pulmonary œdema (Von Behring).

In a report to the German Agricultural Board at Berlin, on February 8, 1906, Von Behring made known his discovery of a new preparation, to which he has given the name **Tuberculase**—this he has successfully used for cattle a year or more old.

No description of the mode of preparation of tuberculase has yet been issued, but it is reported to be a powerful vaccine, containing no living organism, and is prepared apparently from *killed* human tubercle bacilli.

It is a semi-liquid substance, and can be injected subcutaneously without any special precautions.

Von Behring states: 'A great question is that of the prevention of human tuberculosis by the new methods. I have indicated to the Congress at Paris that prophylaxis

(in the human subject) is not realisable by a method analogous to bovo-vaccination in which living bacilli are introduced into the circulation.

'On the contrary, I estimate that vaccination of children is possible by the subcutaneous injection of a substance containing no living bacilli, and which I have designated under the name of tuberculase.'

Vaccination against Tuberculosis by Way of the Digestive Tract.

Calmette and Guérin administered, by means of an œsophageal sound, to two calves 0·05 gramme, followed forty-five days later by a dose of 0·25 gramme, of living **human** tubercle bacilli.

Four months afterwards these two animals, together with another calf (unvaccinated) to act as a control, were all tested with tuberculin. To this they gave no reaction. All three animals were now fed with 0·05 gramme of virulent *bovine* tubercle bacilli.

Thirty-two days later they were again submitted to the tuberculin test.

The control (unvaccinated) calf now gave a marked reaction, whilst the two vaccinated animals remained unaffected.

From these experiments it appears possible to immunise animals by feeding them with tubercle bacilli of slight virulence (human tubercle bacilli non-virulent for cattle).

Calves were immunised also by the administration by way of the alimentary tract of *killed* **bovine** tubercle bacilli.

Experiments conducted at Alfort by Roux and Vallée have given results identical with those of Calmette and Guérin.

The duration of the immunity obtained by the method above described remains as yet unknown.

The Serum Treatment of Tuberculosis.

Many attempts have been made to treat cases of tuberculosis in the human subject with the serum of horses injected with progressively increasing doses of the products of growth of Koch's bacillus.

Amongst the different antitubercular sera so far introduced, the most noteworthy are those of Maragliano and Marmorek. Unfortunately these antisera have not given very encouraging results in the war against tuberculosis.

According to Vallée the failure is explained by the fact that these sera have been solely anti*toxic*.

In the treatment of tuberculosis this authority advocates the association of both antitoxic and antimicrobic sera. Such a serum is obtained by injecting horses first with *all* the toxius of the tubercle bacillus,[1] subsequently vaccinating them with human tubercle bacilli after a method similar to that of Von Behring.

Two calves each received an intravenous injection of virulent *bovine* bacilli. One of these animals died in thirty-

[1] Vallée maintains that up to the present the antitoxic tubercular sera have not been obtained with *all* the toxins of the tubercle bacillus. There exists, he says, in addition to the soluble toxins present in bouillon cultures of the tubercle bacillus, other toxins which remain confined in the interior of the bacilli. The diffusion of these endotoxins into the surrounding culture medium is prevented by the waxy sheath surrounding the microbes. The endotoxins are more toxic than the exotoxins.

In order to obtain all the toxins from the microbes, Vallée treats them by a special process in which the desiccation of the organisms over sulphuric acid, their subsequent grinding, and extraction by means of prolonged agitation with ether in flasks containing glass balls, forms an essential part.

three days, after having shown a temperature above 40° C. for many days prior to death. Post-mortem examination disclosed the presence of extensive lesions of tuberculosis in the lungs.

The other calf, after the initial injection of virulent bacilli, was treated with the antibacterial serum.

This animal, in marked contrast to the control, showed no thermal reaction, and remained to all appearance perfectly healthy. Finally it was slaughtered, and, on post-mortem examination, the carcass was found quite free from lesions of tuberculosis.

According to these experiments the antimicrobic serum is possessed of distinct protective and curative properties.

Behring has also introduced an antitubercular serum, but it seems that the exact details of preparation have not yet been published.

The method of immunising calves against tuberculosis by inoculating them with Von Behring's bovo-vaccin, although apparently effective, is yet a somewhat lengthy process, and whilst undergoing vaccination animals are more susceptible than ordinarily to the usual modes of infection.

In veterinary practice it is here that antitubercular serum may find one field of usefulness in protecting animals temporarily during this period of increased susceptibility to infection.

Glanders.

At various times the treatment of glanders in animals has been attempted by injecting them with the blood-serum of animals immune from that disease.[1]

[1] Bovine animals are possessed of a natural immunity against glanders, *but this immunity is not absolute.* From Riegler and Ciuca's recent experiment it appears that cattle can be experimentally inoculated with glanders, when large doses of virulent cultures of the *B. mallei* are employed.

Babes introduced the blood-serum of an ox, and Prieur speaks with confidence of the treatment of cutaneous glanders in man and certain cases of pulmonary glanders in the horse, by this method.

Malzew claimed to have immunised animals by injecting them with normal ox serum, and Chenot in this way succeeded in curing seven guinea-pigs previously inoculated with glanders, but on three other glanderous guinea-pigs the treatment had no effect. Similar attempts made by Nocard and Leclainche failed.

Cadiot treated animals with defibrinated ox blood-serum and with blood-serum obtained from birds previously inoculated with sterilsied cultures of the *B. mallei* and with mallein. But no success attended this author's experiments, and at the present time there is no known method of immunising animals against glanders.

Swine Erysipelas

(*Rouget du Porc*).

This disease, peculiar to the pig, is not very prevalent in Great Britain, where it apparently takes a chronic form, the most prominent lesion met with on post-mortem examination being verrucose endocarditis. Occasionally, however, outbreaks of acute and rapidly fatal swine erysipelas occur, as in the recently reported cases in Cambridge, resembling the form seen in Germany, France, and Denmark, where the disease appears to be quite common, and the mortality averages 80 per cent. in some districts.

The micro-organism of swine erysipelas is a very fine rod-shaped, non-motile bacillus found in the blood of affected animals, and in the diseased organs, fæces, etc. (see Figs. 1 and 2).

The principal methods of protective inoculation against this disease are :

1. **Pasteur's Method.**—Inoculation with two vaccines of different degrees of virulence.

2. **Lorenz's Serum Method.** — The simultaneous injection of immune serum and virus at different parts of the body.

3. **Leclainche's Method.**—Inoculation with a serum virus mixture, followed in twelve days by the injection of a pure culture of the organism without any serum.

1. **Pasteur's Method.**—It was found that the repeated passage of the swine erysipelas bacillus through pigeons exalts, whilst, on the other hand, its passage through rabbits diminishes, its virulence for swine.

After a time the virus obtained from the rabbits no longer kills swine, but produces only a mild attack of the disease, from which the animals recover and acquire immunity.

The degree of attenuation obtained by passing the organism through rabbits persists in bouillon cultures made from the latter, and these are used to vaccinate swine.

Pasteur made use of two vaccines, injecting first a feeble vaccine, and ten to twelve days later a second more virulent one.

The dose of these vaccines is 0·1 c.c., injected subcutaneously inside the thighs.

It is recommended that whenever possible the operation should be performed on young pigs, as these are less liable than adult swine to contract the disease.

Immunity is established a fortnight or so after the second inoculation, and lasts about one year.

The injection of Pasteur's vaccines gives rise to a febrile reaction more or less intense, and not rarely a high rate of mortality attends this method of immunisation.

2. In **Lorenz's Method** a serum is prepared by infecting animals *already immunised* with large doses of virulent cultures. The pig was first used, but on account of the difficulty in handling this animal resource was later had to larger animals. Lorenz and Leclainche use the horse, whilst Kitt and Schreiber prefer the ox.

The horse furnishes an active serum after treatment for two or three months with gradually increasing doses of culture. Towards the end of the hyper-immunising process $\frac{1}{2}$ litre or more of virulent culture of swine erysipelas bacilli may be injected into this animal without ill effect.

The immune serum, prepared in the manner just described, will, of course, if injected into a susceptible animal (pig), confer on it an immediate immunity against swine erysipelas, but this immunity lasts only for a few days (*i.e.*, it is a temporary passive immunity). On account of this, and in order to insure a more lasting immunity, Lorenz later devised the method of following up the injection of immune serum with a dose of virulent culture.

The serum is injected in the region of one of the ears, and immediately afterwards the virus is introduced, still in the same region, but on the opposite side of the body.

To test the activity of the serum before using it for the purpose of immunising swine various methods have been adopted by different workers :

Leclainche uses the pigeon, injecting a serum virus mixture intravenously. He considers a serum sufficiently active if, when mixed in the dose of 0·5 c.c. with 1 c.c. of culture, it preserves the pigeon against the effects of the virus. Deutsch also employs the pigeon, but his serum protects in the dose of 0·5 c.c. against only the same quantity of culture, the mixture being injected in the breast.

Prettner advises testing the serum on white rats by successively inoculating them subcutaneously with a certain

quantity of serum, and twenty-four hours later with a dose of culture. He considers a serum sufficiently active when it protects the animal in the dose of 0·01 c.c. against the same quantity of culture (*i.e.*, 0·01 equals 0·01).

3. **Leclainche's Method.**—Leclainche advises inoculation with a *mixture* of serum and culture, and after an interval of twelve days with a pure culture of the bacillus.

He recommends that **when the disease has already appeared in a district** the pigs should be given a preliminary injection of serum alone, from 10 to 20 c.c., according to weight. Ten days afterwards they are vaccinated, first with a mixture of serum and culture (1 c.c. serum for every 20 pounds live weight, using a minimum of 5 and a maximum of 10 c.c., culture 0·8 c.c.), and twelve days later with 0·8 c.c. of culture without any serum.

The injections are given subcutaneously inside the thighs or at the base of the ears.

In practice the methods of Lorenz and Leclainche have proved entirely satisfactory.

It is reported that in Belgium 15,329 pigs were immunised against swine erysipelas by Leclainche's method with complete success.

Swine Plague

(*Pasteurellose du Porc, Contagious Pneumonia of Swine*).

This, one of the pasteurella group of diseases, although prevalent in certain Continental countries, is unknown in Great Britain as a separate contagious disease of swine. It may be met with as a complication in cases of swine fever.

Kitt attempted the immunisation of pigs against swine plague by the intravenous injection of the causal organisms previously killed by heat. In his first experiment four rabbits were inoculated with the microbes previously

heated from 52° to 55° C. for twenty hours. Two of these animals resisted inoculation of virus which proved fatal for control subjects in twenty-four hours; the other two survived, one for three and the other for five days after injecting the virus.

Two other series of experiments gave similar results, and two young pigs treated in the same way resisted the ingestion of virulent viscera which killed control animals in a few days.

Experiments have been conducted by a number of workers with a view to obtaining a protective **serum** against swine plague. Such a serum has been obtained from animals treated with the injections of the *B. suisepticus* (swine plague bacillus).

This serum was issued commercially under the name of '**septicidin**,' but no practical value attended its use. Wassermann and Ostertag have explained the cause of failure. They showed that a monovalent serum—*i.e.*, a serum obtained by treating animals with one variety only of the swine plague bacillus—did not exhibit sufficient protective power against other strains or varieties of that organism, but was only capable of protecting against the particular strain of organism which had been used to immunise the animal yielding the serum.

So that *polyvalent* sera are preferable for the purpose of passive immunisation; but at the present time there appears to be no satisfactory method of conferring on swine either an active or passive immunity against swine plague.

(See also Lignière's polyvalent vaccines and sera against the pasteurelloses, p. 49).

Swine Fever

(*Peste du Porc, Hog Cholera*).

Kitt successfully immunised animals by inoculating them intravenously with cultures of the swine fever bacillus pre-

viously heated for four hours at a temperature of from 50° to 53° C. He states that animals treated in this way resist afterwards inoculation with virus fatal for control subjects.

A **serum** method has also been used. De Schweinitz found that a cow inoculated intravenously with gradually increasing doses of virulent cultures of swine fever bacilli furnished a serum possessed of immunising properties. The inoculations of cultures were continued over a period of eight or more months, until 'no reaction followed their injection, and the blood-serum, when added to cultures of the organism, caused agglutination.'

'The serum was further tested as to its power of preserving guinea-pigs inoculated with a lethal dose of living hog cholera bacilli. After separation from the blood the serum was concentrated until it reached a standard at which 10 c.c. proved curative to a pig of 40 to 60 pounds weight. The immunity furnished by such a serum begins immediately, but lasts only until the antitoxin is eliminated from the body; to meet this drawback sterilised cultures (toxins) were inoculated along with the serum' (Law).

These methods of conferring immunity against swine fever are based on the supposition that the causal agent is the *B. choleræ suis*, but Dorset, Bolton, and McBryde have recently shown that there exists in the blood of hogs suffering from acute hog cholera (swine fever) some virus other than that of the *B. choleræ suis*, and this virus is necessary for the production of that disease.

According to these authors **the prime cause of swine fever is the 'filterable virus' (ultravisible virus),** and the bacillus is at most an accessory factor.

In their experiments they found that cultures of the *B. choleræ suis* constantly caused death in hogs when administered intravenously, and usually so when administered

by the mouth, but not, as a rule, when injected subcutaneously.

Whilst the disease (swine fever) contracted by *natural* infection is highly contagious, and the blood of animals sick from natural infection is always infectious for other hogs by subcutaneous inoculation, and, moreover, whilst hogs recovered from a *naturally* contracted attack of the disease possess a high degree of immunity against subsequent infection, all these features are wanting in the disease, produced by the administration of *cultures* of the *B. cholerœ suis*.

On the other hand, 'the experiments with blood-serum derived from hogs sick of hog cholera proven to be free from the bacillus show that such serum produces illness in hogs with great regularity upon subcutaneous injection, and, furthermore, the disease thus produced possesses all the characteristics of the natural disease, including "infectiousness of the blood," and immunity in those animals which recover.'

It is supposed that the swine fever bacillus may be a frequent inhabitant of the intestines of the normal hog.

The filterable virus seems not only to cause disease by itself, but it seems also to be able to lower the resisting power of the hog, and thus enable the *B. cholerœ suis* to invade the body, and this is believed to take place in the majority of cases of natural outbreaks of swine fever.

The Eighth International Veterinary Congress (1905) passed the following resolutions in reference to measures against swine plague (*pasteurellose du porc*) and swine fever:

1. Swine fever (which is often a mixed infection) ought to be controlled by veterinary police and hygienic measures.

2. At the present time no known protective or curative inoculation methods can be recommended. Further careful investigation is necessary to determine whether such methods and materials are to be sought in the directions already indicated, or whether entirely new methods must be devised.

CHAPTER III

DISEASES DUE TO ULTRAVISIBLE VIRUSES

Rabies—Variola—Cow-pox—Sheep-pox—Bovine contagious pleuro-pneumonia — Foot and mouth disease — Rinderpest — South African horse sickness.

In the diseases next to be considered—

Rabies,
Variola,
Bovine contagious pleuro-pneumonia,
Foot and mouth disease,
Rinderpest, and
South African horse sickness,[1]

the actual causal agent has yet to be discovered.

The virus is present in all cases in the diseased tissues, and in some of the above-mentioned diseases (rinderpest and horse sickness) also in the blood of affected animals, for its inoculation into susceptible subjects will reproduce the disease; but the pathogenic agent is beyond the range of vision, even with our most powerful microscopes: it is ultramicroscopic.

[1] It would now appear necessary to include both swine fever and canine distemper in this list of diseases due to an ultravisible virus.

Rabies.

The dog is the most frequent victim to this disease, although it affects all the domesticated animals and is transmissible to man.

The virus exists especially in the saliva and the nervous structures—brain and spinal cord.

Rabies is generally transmitted by the bite of an affected animal; in this way the virulent saliva is readily inoculated.

The period of incubation varies, depending on the species of animal and on the distances of the wound from the central nervous system. From the site of inoculation the virus passes to the central nervous system by way of the peripheral nerve trunks.

In the rabbit, after intracranial inoculation of rabic material, the incubation period is from fifteen to seventeen days. Symptoms of paralysis result; rabies of the rabbit is therefore a form of the so-called 'dumb rabies.'

In the dog the disease manifests itself in two forms—the more common 'furious rabies' and the 'dumb' form.

Pasteur observed that when rabic virus was passed through a series of apes it became attenuated, whilst it increased in virulency after passing through a series of rabbits. He noted from this fact that the virulence of the virus was artificially alterable, and proceeded to experiment with a view to finding a vaccine against the disease.

When the natural virus (such as the brain or spinal cord of a rabid animal) is passed through a series of rabbits—inoculated from rabbit to rabbit—after fifty passages the period of incubation shortens from fifteen or seventeen days down to six days. After this the rabbits die regularly six days after inoculation, no matter to what generation the virus may belong later than the fiftieth.

This exalted virus is known as fixed virus (*virus fixé*), and

is the strongest rabic virus obtainable. It is exalted not only for rabbits, but also for dogs.

Having now obtained a virus of known and fixed strength, one may proceed to attenuate or weaken it until a suitable vaccine is obtained; in Pasteur's method this is accomplished by drying.

A virulent rabic cord *gradually* loses its activity by desiccation in the air. This attenuation shows itself by retardation in appearance of symptoms of the disease after inoculation—in other words, a lengthening of the period of incubation. After drying for fifteen days the cord is inert.

In Pasteur's method of preparing an antirabic vaccine the spinal cord (fixed virus) is used, and after division into segments of 2 centimetres in length, these are suspended in wide-mouthed bottles containing pieces of caustic potash (to absorb moisture from the air) and maintained at a temperature of 23° C.

(The cords are, of course, always obtained from animals of known and definite weight; in Paris $2\frac{1}{2}$ kilogrammes is the standard weight of rabbits used for the purpose.)

In Pasteur's **old method** of antirabic vaccination on the first day of treatment the patient was inoculated with an emulsion of 2 millimetres of cord dried for fifteen days; on the second he received a dose from a fourteen days' cord, on the third from a thirteen days' cord, and so on, until finally a cord dried only for three days was used. The injections were given daily, and introduced subcutaneously.

This method proved ineffective for severe cases, and in consequence a **new system** of dosage was introduced. Cords dried for fifteen days were found to be inert and were discarded, treatment commencing later with those dried for fourteen days.

Different institutions employ somewhat different methods of arranging the injections. The following (after Hewlett) is a tabular arrangement of the method employed at Lille, the dose being 2 millimetres of cord emulsified in sterile broth or physiological salt solution :

ORDINARY TREATMENT.

Day of Treatment.		Days of Desiccation of Cord.
1st.	Morning	14
	Evening	13
2nd.	Morning	12
	Evening	11
3rd.	Morning	10
	Evening	9
4th.	Morning	8
	Evening	7
5th.	Morning	6
6th.	Morning	5
7th.	Morning	4
8th.	Morning	3
9th.	Morning	9
	Evening	8
10th.	Morning	7
	Evening	6
11th.	Morning	5
12th.	Morning	4
13th.	Morning	3
14th.	Morning	9
	Evening	8
15th.	Morning	7
	Evening	6
16th.	Morning	5
17th.	Morning	4
18th.	Morning	3

FOR SEVERE BITES, IN ADDITION :—

Day of Treatment.	Days of Desiccation of Cord.
19th. Morning	7
Evening	6
20th. Morning	5
Evening	4
21st. Morning	3

The German method differs from the above. In Berlin it is considered that the first few days of Pasteur's method of treatment are wasted, quite inert material being injected. (They consider that the desiccated cords are harmless after the eighth day, whereas according to the French observers this state of affairs is not reached until the fourteenth day of drying.) Consequently, they commence with an eight days' cord, and on the sixth day of treatment use a cord dried only for three days. The process is repeated during the continuance of the twenty-one days of treatment. During the final days cords dried for two days only, or slightly less, are injected.

Statistics have proved the value of Pasteur's method.

The treatment, although applicable after the bite of a rabid animal, is really *preventive, and not curative,* since it fails if symptoms of the disease have actually developed.

The Pasteur inoculation undoubtedly protects animals from rabies, the duration of immunity after vaccination lasting in the dog at least three years.

Antirabic Serum.

The **blood-serum** of immunised animals has some action on the virus of rabies.

In the preparation of an **antirabic serum** an animal is

immunised by a process similar to that just described—*i.e.*, by receiving a succession of graduated doses of virus.

The sheep has served almost exclusively for the preparation of antirabic serum. The immunising process is commenced by careful intravenous injections, then continued by subcutaneous. After a time an entire rabbit's brain emulsified in 400 c.c. water may be injected. According to Remlinger, when the sheep has received thirty to forty brains its serum may be used, the blood being drawn ten days after the last injection. The inoculation of a rabbit's brain (virus) each month suffices to maintain the activity of the serum.

Injection of this antirabic serum alone has, of course, only a temporary immunising effect; therefore **a mixture of antirabic serum and virus** has also been used to immunise animals against rabies.

Sero-virus Inoculation.

Marie states that dogs inoculated subcutaneously with a **mixture** of 3·5 c.c. of antirabic serum and 2·5 grammes of fixed virus have afterwards successfully resisted the inoculation of virulent rabic material.

An immunising mixture of one-third virulent emulsion and two-thirds immune serum is usually employed, from 1 to 50 c.c. being injected under the skin of the abdomen in one dose.

This writer finds that injection of the virus-serum mixture is able to immunise dogs against the action of rabic virus fatal for controls, and the immunity so conferred lasts for ten to twelve months.

Marie concludes: 'This method confers a rapid immunity, whereas the Pasteur method of vaccination necessitates the attendance of the patient for some fifteen days, and only after a long series of injections is the resistance of the animals established, whilst the treatment now proposed

protects animals three days after inoculation against infection of virus, even into the anterior chamber of the eye, and the intra-ocular injection is a test more severe than the most serious bite.

Variola.

Variola (small-pox) affects man and all the domesticated animals—*Variola vaccinia* (cow-pox), *V. equina* (horse-pox), *V. ovina* (sheep-pox), *V. caprina* (goat-pox), *V. suilla* (swine-pox), *V. canina* (dog-pox), etc.

With the exception of sheep-pox, the variolous diseases of the lower animals are usually benign, of rare occurrence, and seldom require treatment. One attack confers on the subject immunity against a second.

Bollinger held the opinion that sheep-pox and small-pox were the two primary forms of variola, and all the other forms (cow-pox, horse-pox, etc.) were secondary varieties, and had been carried from man or from sheep to other animals.

'The close relationship of these various kinds of variola is proved by their reciprocal power of transmitting the affection and of conferring immunity. Their respective identity with small-pox is amply demonstrated by the frequent interchanges made between small-pox and animal variola' (Friedberger and Fröhner).

In particular the virus of cow-pox (vaccinia) is generally considered to be a modified form of the small-pox virus of man, which becomes attenuated in virulence on its passage through the cow. **The inoculation of man with vaccine virus confers on him an immunity against small-pox.**

Hewlett considers that 'arm-to-arm vaccination is undoubtedly the most efficient form of inoculation against small-pox, but this method is no longer practised, owing to the risk of conveying such diseases as syphilis. For it calf lymph has been substituted.'

Calf Vaccine ('Vaccine Lymph').

For the preparation of vaccine lymph calves are used, generally those of from three to six months old being selected for the purpose.

The calf is inoculated with vaccine lymph from a previously vaccinated calf, the region selected for the operation being the lower surface of the abdomen or abdomen and thorax.

After a careful examination to see that it is perfectly healthy, the animal is placed on a special operating-table, the area to be vaccinated shaved, washed with a 5 per cent. carbolic solution, then with sterilised water to remove every trace of the disinfectant, and finally thoroughly dried with sterilised cotton-wool swabs.

Next a series of lines are scarified by means of a sterilised scalpel, penetrating only through the epidermis, and avoiding hæmorrhage as far as possible. The lines are continued in parallel rows along the surface of the vaccination area, the distance between them being usually about 1 inch.

After the resulting slight hæmorrhage has ceased, a sufficient quantity of a previously prepared glycerinated vaccine (stored for two months before use) is run into each incision.

The animal is now removed from the table and stalled in a shed, means being taken to prevent injury to the surface operated upon.

From the third day following inoculation the scarified lines become very prominent; on the fifth day exudation commences, and from the fifth or sixth to the seventh a large quantity of vaccine lymph may be collected.

In summer, vaccine may be collected on the fifth day, and in winter even up to the eighth; but if at any time there is the least sign of suppuration in the vesicles the lymph is unfit for use.

FIG. 27.—CALF 120 HOURS AFTER INOCULATION BY LINEAR INCISION WITH VACCINE LYMPH.

With permission from 'A Manual of Veterinary Hygiene,' by Colonel F. Smith, C.M.G., F.R.C.V.S., etc.

To collect the lymph the calf is again placed on the table, the inoculated area is cleansed with water previously boiled and allowed to cool, then dried, and after loosening the crusts covering the inoculation wounds, the latter are scraped with a small curette.

The base of each swelling is then grasped with a small clamp (to prevent hæmorrhage), and a further quantity of lymph collected.

All the material obtained in this way is mixed together and triturated in a special machine, a quantity of neutral glycerine (or a mixture of glycerine and water) being added thereto. It is next stained through a cloth and received into sterilised glass tubes; these are sealed and stored in a cool, dark place.

From this glycerinated pulp samples are taken from time to time, and by making plate cultivations these are tested to determine the presence or otherwise of extraneous organisms, amongst which the pyococci are the commonest.

After storage for two months, as a rule the lymph is pure, all extraneous organisms having by that time lost their activity owing to the presence of the glycerine. (Although the glycerine kills off these organisms, it seems to have but little effect on the contagium of vaccinia.) When the extraneous organisms have been destroyed, as evidenced by the absence of colonies in the agar plates, the vaccine lymph is introduced into sterilised fine capillary tubes; these are sealed in the flame of a spirit-lamp or gas-jet, and finally issued for vaccination purposes. But prior to this the calf which served for the preparation of the lymph is slaughtered, carefully examined post-mortem, and if the carcass is found to be diseased, the lymph from that animal is discarded.

Prepared in the above-described manner, and stored under suitable conditions, the lymph may be kept before

use for three or four months from the time of its collection from the calf; after that length of time it gradually loses its activity.

Recently Guérin has shown that—

1. **The rabbit may, equally with the heifer, be used as a vaccine-producing animal.**

2. The rabbit is very susceptible to vaccine virus, especially when the inoculations are made by friction on a freshly prepared skin.

3. **Passage through the rabbit regenerates the virulence of a weakened vaccine.**

4. Inoculation into the peritoneum of this animal, prepared by an injection of sterilised bouillon, enables one to collect, after several hours, an **absolutely purified vaccine** from the peritoneal cavity.

5. Inoculation of a suitably standardised dilution of various vaccine pulps into the rabbit enables one to determine, according to the importance of the eruption obtained, the richness of virulent elements in these pulps.

6. **The testing of vaccines** of bovine origin in the rabbit enables one to select those viruses which seem to be most active.

Variola Ovina

(*Sheep-pox*).

'If it were possible to give immunity against sheep-pox by inoculation with the exudate of cow-pox, and without danger to the sheep from fatal cow-pox, or from its transformation into the more destructive sheep-pox, it would be a very desirable resource. But experiment goes to show that vaccination is useless—in the temperate climates at least' (Law).

Vaccination has been practised by a number of veterinarians in different countries with varying results: some

observers state that inoculation with vaccine from the cow or calf protects against sheep-pox virus; others have come to a contrary conclusion.

In general, for sheep-pox this method of inoculation has been abandoned in favour of **ovination**. This consists in inoculating the animals with the disease sheep-pox in a mild form; in this way the malady is quickly passed through a herd, the outbreak is terminated, and the animals rendered immune against future attacks.

Soulié prepares a **virus** for inoculation purposes in the following manner: A sheep is shorn, shaved over the chest and flank, and the skin on these regions washed and disinfected. Virus from a case of sheep-pox is next inoculated into the skin with a syringe at several different points.

About the twelfth day after inoculation large vesicles form, and from these the virus is collected into sterilised glass pipettes. If stored in a cool, dark place it will retain its activity for at least three months. When required for use, it is diluted in ten times its bulk of a 2 per cent. boric acid solution.

For the purpose of inoculation (ovination) a single scarification is made at the tip of the ear or tail with the point of a lancet holding a small quantity of virus.

The reaction after inoculation continues for about eighteen days, and immunity lasts for a year or more.

The mortality from inoculation is greatest amongst young lambs and pregnant ewes.

Bose has recently introduced a **virus-serum method**; the serum is prepared by hyper-immunising *recovered* sheep by injecting them with large quantities of active sheep-pox virus.

For the immunising process the virus is inoculated as usual at the ear or tail, and 10 to 15 c.c. of serum are injected under the skin at the same time.

In this way fatal results rarely follow inoculation, and a lasting immunity is conferred against sheep-pox.

Bovine Contagious Pleuro-Pneumonia

(*Lung Plague, Lung Sickness*).

This disease, peculiar to cattle, is characterised by pronounced changes in the lungs and pleura of affected animals.

One attack confers immunity ; for this reason inoculation has been practised for many years, the object being to infect the animal with a mild and local form of the disease, from which it may recover.

The material used for inoculation purposes is furnished in abundance by a case of the disease. An animal recently dead from pleuro-pneumonia is selected, and a portion of the infiltrated interlobular pneumonic tissue incised with a clean scalded knife, when a quantity of clear yellow serum flows out. Or, preferably, a puncture is made in the enormously thickened interlobular tissue with a flamed or scalded knife, and flamed sterile pipettes introduced to collect the serum, which readily enters the tubes, filling the vacuum caused by the heat in sterilisation. After the tubes are filled with the fluid they are carefully withdrawn from the lungs and their points sealed in the flame of a spirit-lamp.

Nocard, after washing the surface of the infiltrated lung with boiled water, cut out a deep segment with a sterilised knife, leaving a conical cavity which soon filled with the draining exudate in a pure condition, and this he collected by means of pipettes.

In place of the infiltrated lung the pleuritic effusion from a case of pleuro-pneumonia may be used for the purpose of inoculation.

Pasteur, with ordinary pleuro-pneumonia virus, inoculated

calves subcutaneously behind the elbow or in the region of the dewlap; an enormous engorgement followed from which was obtained a plentiful supply of virus.

For the purpose of inoculation the virus should be used as soon as possible after collection by one or other of the above described methods, since it is undoubtedly weakened by the addition of preservatives. As a preservative glycerine may be added to the virus to the extent of from one-third to one-half its volume; this will insure its preservation for several days.

Method of Inoculation.—This consists in injecting subcutaneously near the tip of the tail about $\frac{1}{2}$ c.c. of virus. As an alternative method a woollen thread previously soaked in the virus may be introduced as a 'seton' an inch or so under the skin of the tail and left there a few days.

The serum collected from the local reaction (swelling) in the inoculated tails may be used to inoculate other animals, and carried indefinitely from tail to tail.

'A local reaction the size of a hen's egg suffices to give immunity, but the greater the local reaction (within limits), the greater the amount of protection. When there is no reaction in an inoculated susceptible animal there can be no immunity.'

'Immunity does not commence until towards the end of the reactionary period, and animals exposed to infection before this time will contract the disease in spite of inoculation.'

After inoculation immunity lasts about one year.

It is advised that 'on the fourth or fifth day following inoculation the animal should be inspected morning and evening; the least sign of carrying the tail away from the body indicates that all is not going right, and demands immediate attention.'

'The only untoward results of the operation are swelling

of the tail at its root, with great pelvic œdema; such cases ought to be recognised before the swelling has extended to the root of the tail, and dealt with by immediate amputation above the seat of inoculation' (Colonel Smith, 'Veterinary Hygiene').

Bouley, Sanderson, Degive, and others, tried injections of virus directly into the jugular vein; by this means no local reaction followed, although immunity was still secured.

This method, however, entails the possibility of producing thrombosis, and considerable danger from the possibility during the operation of introducing the virus into the connective tissue surrounding the vein, and this may entail a fatal issue. The method offers no advantages over those already described. Subcutaneous inoculation by means of woollen threads soaked in the virus has been extensively practised in Africa and Australia; this method offers the advantage of extreme simplicity, and is usually attended with good results.

Foot and Mouth Disease.

Foot and mouth disease occurs chiefly in cattle, sheep, goats, and swine, less frequently in horses, dogs, cats, and birds, and is readily transmitted to man.

An animal may suffer several times from the malady; one attack does not confer an immunity lasting for any considerable length of time; consequently, protective inoculation is hardly justified.

For the purpose of cutting short an outbreak and passing a whole herd promptly through the malady inoculation has been practised in Germany and other Continental countries.

The usual method of carrying out this process was either (1) by placing some of the saliva from an animal affected with a mild form of the disease into the mouth of the one

to be inoculated by means of a wisp of hay, after slightly excoriating the mucous membrane; or (2) inoculating it subcutaneously with a lancet. Animals so inoculated are only relatively immune for a period of a few months (about three), so that the operation is of doubtful value.

A **serum method** has been introduced within the last few years, and under certain conditions (see pp. 43 and 44) a preventive and curative serum could be utilised with advantage.

Such a serum is prepared by treating animals which have recovered from a severe attack of the disease with injections of large doses of virus (contents of vesicles). Horses treated with increasing quantities of virulent lymph furnish a serum which, when administered in doses of 5 to 20 c.c., according to the size of the animals to be injected, will preserve them from the malady, but, as in the case of all the antisera, the immunity so conferred is but temporary (passive), lasting only for a period of two weeks.

Löffler has introduced a method of active immunisation by injecting mixtures of prophylactic serum and virulent lymph. Immunity is established about three to four weeks after the inoculation, and is equal to that acquired after recovery from the disease when naturally contracted.

Rinderpest

(*Cattle Plague*, *Bovine Plague*, *Bovine Pest*).

As the name indicates, this is essentially a disease of cattle, although other ruminants are also subject to the malady—goats and sheep especially. The blood, fæces, and the secretions and excretions of an animal suffering from the disease are all extremely virulent. It is attended with a high rate of mortality, but animals which recover

from one attack are immune against subsequent infection. Horses, dogs, birds, and man are naturally immune.

The principal methods of immunising animals against rinderpest are—

1. By inoculating them with the bile of cattle which have succumbed to rinderpest. The bile alone may be used, or mixtures of bile and glycerine.

2. By injecting the serum or defibrinated blood of immunised animals.

3. By serum-virus inoculation.

1. **The Bile Method.**—For the purpose of immunisation bile is taken from the gall-bladder of an animal immediately after it has succumbed to rinderpest.

Preference should be given to biles of a dark green colour. Putrid biles, or those red-coloured from the presence of blood, must not be used.

Koch, who introduced this method of immunisation, believed that the organism of rinderpest present in the bile was restrained by the immunising substances also contained therein. The proportion of virus and immunising substance present in different biles is subject to variation, and therefore this method is somewhat uncertain in its effects. It may induce the disease, in subjects especially susceptible, it may be in a fatal form, but this danger is obviated by storing the bile for forty-eight hours before use, or by adding to it glycerine.

Pure bile should be utilised within four days from the time of its collection; otherwise, unless preserved by the addition of glycerine, it appears to become inert.

About ten days after inoculation of bile immunity is established, and this lasts only four months on an average.

The dose of bile has been fixed at a minimum of 10 c.c. It should be injected subcutaneously in the region of the 'dewlap.'

Edington's Glycerinated Bile is a mixture of 1 part of glycerine and 2 parts of bile. After mixing, this is allowed to stand forty-eight hours or longer before being used for the purpose of inoculation.

Whilst bile alone will not 'keep' for any length of time, glycerinated bile may be kept for months. Hutcheon reports that in one instance it proved effective after three years' storage!

As an immunising agent glycerinated bile is decidedly safer than fresh, pure bile—in fact, large doses of the mixture may be injected intravenously without danger. On the other hand, it shows more feeble and but temporary immunising power; consequently, it is advisable, ten days or so after its administration, to inject a dose of fresh bile without glycerine.

Says Hutcheon: 'Glycerinated bile is now used mainly to give a temporary immunity similar to serum, until the infection in the herd or in the near neighbourhood has died out. If necessary, it may with advantage be followed by a dose of pure bile. It is now rarely followed by any virulent blood, principally on account of the danger that exists in this country (Cape Colony) of communicating other diseases besides rinderpest.'

The dose of glycerinated bile—either subcutaneously or intravenously administered—is from 15 to 30 c.c., according to the size of the animal.

2. **Anti-Rinderpest Serum.**—A protective and curative serum is obtained from animals actively immunised against rinderpest. An ox already immune against the disease—either by a natural or artificial process—is injected periodically with gradually increasing doses of virulent rinderpest blood, until finally it will withstand a dose of 1,000 c.c., or more.

For protective purposes the serum is injected subcutaneously in doses varying from 50 to 200 c.c.

The immunity induced by serum lasts only eight to ten days.

In making use of the 'serum alone' method it is necessary to inoculate all susceptible animals likely to come in contact with the infection; moreover, strict isolation of the infected herd must be rigidly enforced. All movement of animals either into or out of the infected area must be prohibited until the outbreak is at an end.

Stockman affirms that three repetitions of serum injected at intervals of ten days will suffice to protect animals during the course of an outbreak—*i.e.*, they are immune for a period lasting over thirty days—a period long enough where strict isolation is enforced and movement prevented to allow all ordinary sources of infection to die out.

The **defibrinated blood** of animals immune against rinderpest has also been used for inoculation purposes, but on account of the danger of transmitting other diseases (piroplasmosis, etc.), it has now been abandoned in favour of serum.

3. **Sero-virus Inoculation.**—Turner and Kolle introduced into South Africa the method of rendering cattle actively immune against rinderpest by inoculating them *simultaneously* in different parts of the body with 1 c.c. of virulent blood and a dose of immune serum—the dose of the latter varying from 15 to 40 c.c., depending upon the strength of the particular serum used.

After the initial dose of serum, other methods of introducing the virus have been practised by different workers in this field. Briefly, these are—(*a*) By 'drenching' the animal with virulent blood; (*b*) by smearing on the nose of the subject either virulent blood or excreta from an affected animal; and (*c*) by placing the animal in contact with others actually suffering from rinderpest.

Of these different methods, that of Turner and Kolle

has been extensively practised; they all have one object in common—namely, to induce in the subject a mild form of the disease, from which it may recover and acquire immunity against subsequent infection. It follows that wherever the sero-virus method is practised, there, of necessity, must be established a new centre of disease; moreover, the operation has not infrequently been attended with a high rate of mortality.

Finally, there exists the danger of introducing with an injection of virulent blood the germs of other diseases, such as piroplasmosis.

It is in countries in which the disease is already well spread, and where it is impossible adequately to control the movement of animals, that sero virus inoculation against rinderpest may be indicated.

A Veterinary Congress held in Bloemfontein, December, 1903, passed the following resolution:

'The means best suited to attain this end' (*i.e.*, the eradication of rinderpest) 'are the stamping out of outbreaks by a liberal use of serum, if obtainable; failing this, pure bile inoculation carried out under professional supervision is preferable to any other method.'

South African Horse Sickness.

This disease affects in order of susceptibility the horse, mule, and ass, and is easily inoculable from an affected to a susceptible animal.

It is due to an ultravisible virus which is able to pass through the finest Chamberland filter. The blood of affected animals is always virulent, so much so that when injected into susceptible subjects in doses of 1 c.c., or less, the disease is unfailingly reproduced.

The virus appears to lose its activity on drying, but against all other agents it is extremely resistant.

Animals which recover from horse sickness acquire immunity, but they may have relapses.

Theiler finds that the serum of an animal which has recovered from the disease has no preventive value; but the serum of an animal which has recovered from horse sickness, and been afterward hyper-immunised by the periodical injections of virus, has a preventive action—(*a*) when mixed with virus; (*b*) when injected before virus; (*c*) when injected in large quantities simultaneously with virus.

Stockman summarises the results of the experimental work on this subject as follows:

1. If 1 c.c. virulent blood and 50 to 100 c.c. serum be mixed and injected subcutaneously, the animal so operated upon does not take horse sickness, but acquires immunity from the operation.

2. The same mixture, when injected into the jugular vein, causes death from horse sickness.

3. Serum alone injected under the skin gives immunity for about a month. If the virulent blood be afterwards inoculated within the period of resistance the animal survives, but does not acquire *active* immunity. *A reaction must take place to produce the latter condition.*

4. Simultaneous injection *under the skin* at different places of 1 c.c. virulent blood and 100 c.c. serum gives no result other than passive immunity, *but if the virulent blood be injected into the veins at the same time instead of under the skin a reaction always follows. The virus gets far enough ahead of the serum to produce a reaction.*

The minimum amount of serum necessary to protect against a fatal issue is 200 to 350 c.c according to live weight.

The immunity so acquired is active.

Says Theiler: 'The simultaneous injection of virus intra-jugularly and serum subcutaneously, followed by an injection of serum within the incubation period (with the object of modifying the horse sickness reaction), produced the most satisfactory results.

All mules which recovered from the reaction proved to be immune when tested with virus or when exposed to natural infection.

CHAPTER IV

DISEASES CAUSED BY THE PROTOZOA

Intracorpuscular parasites—The piroplasmoses—Texas fever—Red water—Rhodesian red water—Equine biliary fever—Canine malignant jaundice — Extracorpuscular parasites — Trypanosomiases — Surra — Nagana — Dourine — Mal de caderas — Gambian horse disease—Gall sickness—Spirochætes—*Spirochæta anserina—Spirochæta Theileri—Spirochæta gallinarum.*

THE diseases considered in the preceding pages are caused either by—

(1) Bacteria, bacilli, cocci. These are organisms belonging to the **vegetable** kingdom. Or,

(2) A virus ultramicroscopic. In this case the causal agent is beyond the range of vision with any of our modern microscopes, and in many cases is so extremely small that it will pass through bacteriologic filters, the filtrate still retaining its virulency for susceptible animals. To this variety of organism it is, of course, impossible to assign any accurate classification beyond the term 'ultravisible virus.'

But there is still another group of infective diseases—diseases produced by the presence in affected animals of micro-organisms belonging to the **animal** kingdom, and visible microscopically at least during some portion of their life-cycle.

Conveniently for the purpose of description we may roughly divide these animal micro-organisms into two groups:

1. **Intracorpuscular.**—Parasites present in the interior of blood-corpuscles at any rate during the greater part of their existence in the animal host. They may, however, at some period of their life-cycle be liberated from the corpuscles and set free in the plasma.

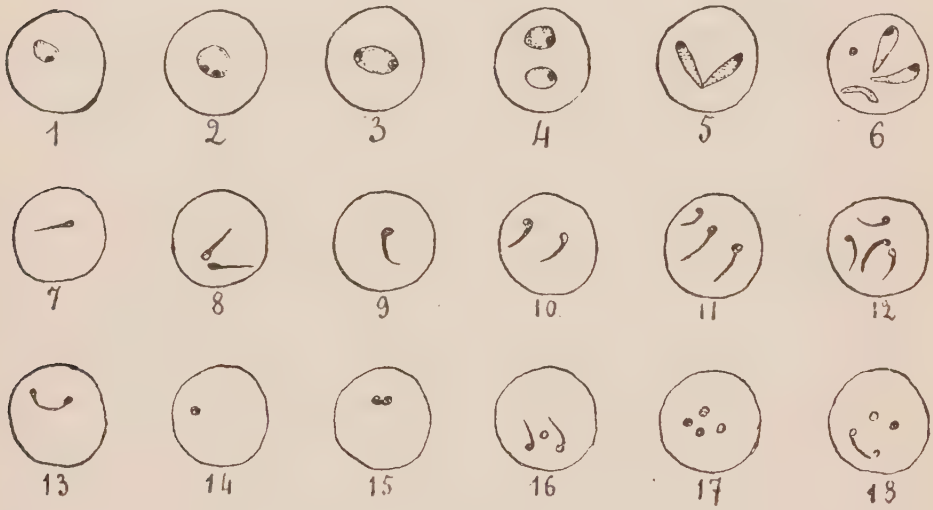


FIG. 28.—AFTER LAVERAN.

Nos. 1 to 5, Typical forms of *Piroplasma bigeminum* in erythrocytes; No. 6, a red blood-corpuscle containing both a typical and an atypical form; Nos. 7 to 18, atypical forms (× 1,800).

This class includes the malarial organisms of man and the piroplasmata of animals.

2. **Extracorpuscular.**—Parasites present in the blood or other body fluids. They are not found in the interior of blood-corpuscles, but remain free in the blood-plasma and extra cellular fluids.

This group includes the trypanosomata and spirochætæ.

GROUP I.: INTRACORPUSCULAR PARASITES (PIROPLASMATA).

These small parasites occurring in the red blood-corpuscles are usually either round or *pear*-shaped—hence the name *piro*plasma. The pear-shaped organisms—associated as a rule in one or more pairs—result from the division and subsequent drawing apart of the ring-shaped forms. Intermediate stages between the ring and pear may be seen (Fig. 28, Nos. 1 to 5).

These are the **typical** piroplasma, but they occasionally present other and **atypical** appearances—'coccus-like,' bacillary, rod, or baton shape, etc. (Fig. 28, Nos. 7 to 18); especially are these found in the disease Rhodesian red water or African East Coast fever.

If a little fresh-drawn blood containing the piroplasms be placed on a warm stage and microscopically examined, some of the organisms may show amœboid movement.

After staining, preferably with eosin methylene blue, after Romanowsky's or some similar method, it is seen that the parasite consists of a blue stained mass of protoplasm containing a small red-coloured mass of chromatin.

The **piroplasmoses**, or diseases of animals produced by the piroplasmata, are included in the following list :

1. The **Piroplasma bovis**, or *P. bigeminum* is found in cattle, and gives rise to the disease red water, Texas fever, Australian tick fever, or tristezza.

2. **Piroplasma equi** occurs in horses, asses, and mules in the disease bilious fever of Africa, India, etc.

3. **Piroplasma ovis**, seen in the disease of sheep known as 'carceag' in Roumania.

4. **Piroplasma canis**, observed in dogs in the course of the malady 'malignant jaundice' in Cape Colony, Hungary, France, etc.

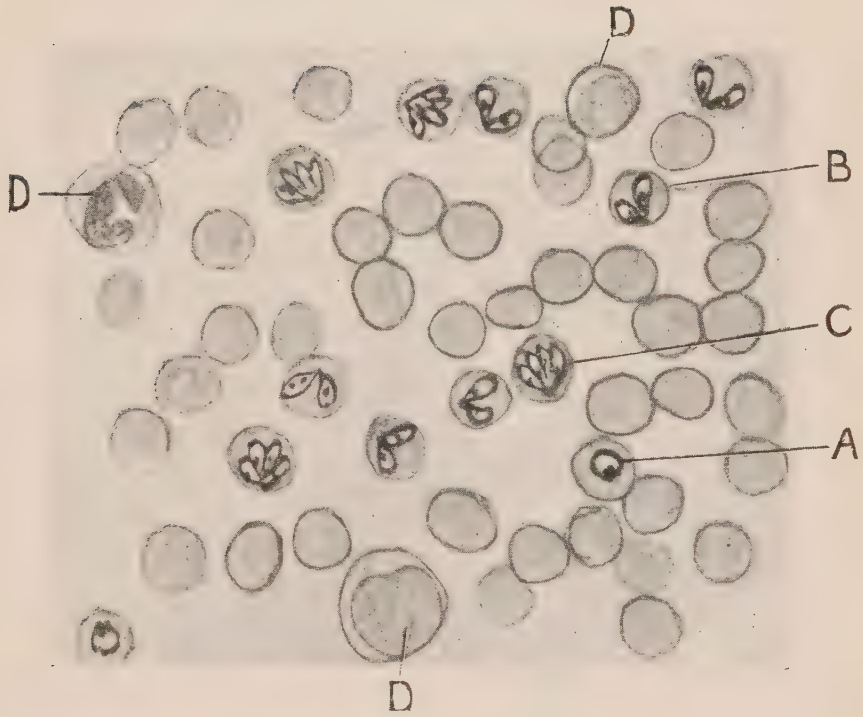


FIG. 29.—PIROPLASMA CANIS.

A, Erythrocyte containing spherical piroplasma; B, pear-shaped and oval forms; C, red blood-corpuscle enclosing four parasites; D, leucocytes.

Drawn from blood-smear stained by Romanowsky's method. Composite field Leitz $\frac{1}{16}$-in. oil-immersion objective. Drawing eyepiece.

In appearance all the above-mentioned micro-organisms resemble each other fairly closely, but the *P. equi* and *P. ovis* are smaller than either the *P. bovis* or *P. canis;* and, again, whilst in bovine piroplasmosis it is unusual to find in one red blood-corpuscle more than one or two parasites (bigeminum), in canine piroplasmosis four are quite commonly observed, and sometimes as many as eight or even sixteen may be seen in one erythrocyte.

Although the different piroplasmata in appearance bear a close resemblance to each other, yet their pathogenic action is strictly limited to the particular species of animal in which they naturally occur; thus *P. canis* is pathogenic only for the canine species, *P. equi* for equines, *P. bovis* for bovines, and so on.

5. **Piroplasma parvum**, or **bacillary piroplasma**, produces the disease African East Coast fever or Rhodesian red water. This differs in many respects from the other piroplasmata, as will presently be shown.

The natural mode of transmission of all these parasites from one animal to another is by the agency of ticks, but their life-cycle in the bodies of these intermediate hosts is as yet but imperfectly understood.

Theiler has classified the animal piroplasmoses into two distinct groups:

1. A group in which the parasite *can be inoculated experimentally* with the blood from an affected animal into others of the same species. To this group belong—

(*a*) **Texas fever**, or **red water of cattle**, a disease characterised by anæmia and hæmoglobinuria, and caused by the presence in the red blood-corpuscles of affected and recovered (and consequently immune) animals of the *P. bigeminum.*

(*b*) **Biliary fever** of horses, asses, and mules, and **malignant jaundice** of the dog, caused respectively by

the *P. equi* and *P. canis*, and characterised by profound alteration of the blood, urine, etc.

In both these diseases recovery is followed by immunity, yet, as in Texas fever, the blood remains virulent, for its inoculation into fresh susceptible animals will at any time produce in them the typical disease.

But 'immune blood' from these two diseases (bilious fever of horses and malignant jaundice of dogs) differs from that of Texas fever, inasmuch as in the former the virus —piroplasma—takes an ultravisible form, whereas in the latter—Texas fever—it remains more or less microscopically visible in the red blood-corpuscles of recovered animals.

2. **African East Coast fever** of cattle **(Rhodesian red water)** is caused by a microscopically visible piroplasma, but this is much smaller than the *P. bigeminum* of Texas fever, appears as round and baton-shaped bodies, and differs markedly from Texas fever in that—

1. The causal parasite (*P. parvum*) is found only in the blood of cattle actually suffering or dead from East Coast fever, and *not* in those which recover from an attack of that malady.

2. Experimental inoculation of the blood from affected to susceptible animals does **not** produce the disease.

3. Affected animals show no diminution in the number of red blood-corpuscles, and no symptoms of hæmoglobinuria.

4. Animals recovered from Texas fever are still susceptible to East Coast fever, and *vice versâ*.

In this disease the piroplasma—**Piroplasma parvum**—is transmitted from animal to animal by the agency of ticks, *and in no other way*, whilst in the piroplasmoses previously mentioned the organism is **naturally** conveyed by ticks, but artificial transference of blood containing the organism is equally effectual in reproducing the disease.

Animals having suffered and recovered from one attack

of piroplasmosis are under all ordinary circumstances immune against a second. For African East Coast fever this is true—here the immunity is permanent. In the case of equine bilious fever, whilst recovery secures to the subject a considerable degree of insusceptibility against a second attack, yet the immunity is not *absolute*, since recovered equines do occasionally suffer from a second (though usually mild) attack of piroplasmosis; the same is true for canine piroplasmosis, and in the case of cattle for ordinary red water (Texas fever). 'Cattle known to be immune against red water are often observed to suffer a second time from this disease when weakened through adverse circumstances' (Theiler).

Animals indigenous to old centres of infection invariably show much less susceptibility than those recently imported. It is amongst the latter that the disease, be it Texas fever, African Coast fever, bilious fever, or malignant jaundice, occurs with most frequency, and these supply the majority of fatal cases.

Texas Fever

(*Red Water*, *Australian Tick Fever*).

The methods of immunising cattle against Texas fever are—

1. By inoculating them with graduated doses of virulent or immune blood.

2. By exposing young cattle to the attacks of a limited number of infected ticks.

The effect in both cases is the same—*i.e.*, to induce a mild form of the disease, so that the animal may recover therefrom and acquire immunity. The first method is preferable, as by inoculating with a syringe the dose can be more accurately gauged than the amount introduced by ticks.

Calves, whilst living on milk diet, are immune against

Texas fever; consequently, by exposing such animals to the bites of a few ticks from older cattle they acquire a more permanent immunity, and without danger during the process.

Young cattle are less susceptible than adults; therefore the immunising process should always be practised on these when possible.

Injection of graduated doses of blood containing the piroplasma from an animal which has passed through the disease is practised to a considerable extent in America. As we have already seen, such blood still contains the micro-organism, though it may be in a less virulent form; nevertheless, if an excessive dose be injected, or the subject be especially susceptible, a fatal issue may result from the operation, the fatalities varying from 3 to 10 per cent. This proportion, however, is very small when compared with the deaths amongst untreated animals taken into infested districts—about 90 per cent. on an average.

The operation is simple. Blood is drawn from the jugular vein, defibrinated, and injected subcutaneously in carefully-regulated doses into the animals which it is desired to immunise.

'The dose and number of injections vary with individual animals. As a rule it may be stated that 1 c.c. should be injected into an old animal coming into the infested district, 2 c.c. for a two-year-old, and 3 c.c. for an animal nine to fifteen months old. It will be observed that, unlike the usual custom of applying treatment, the older animals take less than the young ones, owing to their greater susceptibility.

'Where an animal has reacted well to a first injection, and shows a very high temperature, great anæmia, or other symptoms indicative of reaction, it will not be necessary to repeat the injection, but in those cases where the reaction is slight a second injection should follow after an interval of forty days, and, if need be, a third injection after a

similar lapse of time, always increasing the size of dose 50 per cent.

'Usually, after three to ten days, sometimes longer, the inoculated animals show a mild type of Texas fever, which runs a course of from six to eight days, and is followed in about thirty days after the injection with a second attack of a milder character than the first.

'After forty days, when the animal has entirely recovered from the inoculation, a second injection may be given to increase its immunity' (Mohler).

In some cases a very severe type of fever follows the first inoculation, and in about thirty or forty days this is succeeded by a secondary milder reaction.

Immunity is not obtained until some time after inoculation, so that infected ticks should be prevented from reaching the cattle until sixty days thereafter, or until they have quite recovered from the reaction. Once this has occurred a few ticks may be placed on them to reinforce their immunity.

Mohler obtained the best results from his immunising experiments in cool weather and with young cattle, and recommends that animals from six to fifteen months old be selected for inoculation.

Instead of making use of blood drawn direct from an immune animal, Dalrymple has utilised the blood in the bodies of mature engorged ticks found on such animals. Before using them the ticks were washed, and then broken in a mortar and mixed with a few cubic centimetres of sterilised water, and the fluid so obtained used for the purpose of inoculation. Three to twelve ticks formed one dose.

By this method a mild attack of red water (Texas fever) is induced, and after recovery therefrom the subject acquires immunity. It is stated that the effects are equal to those resulting from an injection of blood drawn direct from the vein of an immunised animal, but by the last-mentioned method one can regulate the dose

with more nicety, and there is less danger of organismal contamination. Nevertheless, when it is desired to send some considerable distance material (blood) for the purpose of immunising animals against red water, ticks offer a useful medium for its transmission, since it has not been found possible otherwise effectually to preserve the virus for any considerable period of time.

Rhodesian Red Water

(*African East Coast Fever*).

Koch introduced a method of protective inoculation against this disease. In his third report he states: 'I think that our experiments indicate that the best results may be obtained in protecting against African Coast fever by using freshly-drawn defibrinated blood of recovered animals, and inoculating animals which it is desired to protect subcutaneously with a 10-c c. dose.

'These injections should be repeated four times, with an interval of seven days between the injections. Subsequently the inoculations should be continued, lengthening the intervals between the doses to two weeks, and later to a month, the dose remaining the time at 10 c.c.'

This method has been tried in Africa, but did not meet with much success; as pointed out by Smith, 'if not impossible, protective inoculation is at present outside the range of practical prophylaxis, as the length of time required (viz., five months) is so great that all the animals would be dead on an infected farm before they could obtain immunity.'

The South African Conference of Veterinary Surgeons at Cape Town, on May 25, 1904, passed the following resolution: 'This Conference, after considering the reports of the scientists who have had practical experience of the effects of inoculation as proposed by Dr. Koch, is reluctantly compelled to conclude that it will be vain to

trust to inoculation to arrest the spread of African Coast fever.'

Biliary Fever (Equine Piroplasmosis).

Theiler, who has studied this disease in the Transvaal, summarises the following conclusions:

1. The piroplasma found in the mule and donkey is identical with the *P. equi* found in the horse.

2. The disease caused by this piroplasma is inoculable with blood of immune animals into susceptible ones belonging to the domesticated species of the genus equus.

3. The horse shows the greatest susceptibility for the piroplasma, the donkey is less and the mule the least susceptible.

4. The mule can be safely inoculated with immune blood of any of the three respective equines; the immune horse blood produces the severest reaction; the immune mule blood causes little reaction, and so does the immune donkey blood.

For practical purposes the inoculation of mules with blood of immune donkeys may prove successful.

5. The donkey proves to be equally susceptible to immune blood taken from horses and donkeys, and shows the slightest reaction to the injection of immune mule blood.

For practical purposes the inoculation of donkeys with blood of immune mules may prove successful.

6. The horse, which is extremely susceptible to piroplasmosis due to the injection of immune blood from the horse and mule, seems to suffer less from the inoculation of immune donkey blood.

For practical purposes the inoculation of horses with blood of immune donkeys would have to be taken into consideration.

Malignant Jaundice (Canine Piroplasmosis).

Theiler, as a result of his observations on this disease, summarises the following conclusions :

1. A dog which has recovered from an attack of piroplasmosis has acquired immunity against the disease.

2. The blood of an immune dog acts as virus when injected into a susceptible dog.

3. By hyper-immunising dogs with blood of dogs suffering from piroplasmosis the **serum** acquires preventive properties.

4. The **blood** of a hyper-immunised dog is pathogenic when injected, either defibrinated or non-defibrinated, into susceptible dogs.

5. The **serum** of a hyper-immunised dog is preventive against the *P. canis* of the same dog when injected into susceptible dogs.

6. The serum contains a preventive substance which is not destroyed at 55° C. It is therefore of a complex nature.

7. The mechanism of the production of a preventive serum in an immune dog seems to follow the same laws as exist in producing an antibacterial serum, with the essential difference, however, that the blood of a highly immunised dog remains infective.

GROUP II.: EXTRACORPUSCULAR PARASITES (TRYPANOSOMES AND SPIROCHÆTES).

The Trypanosomata.

These are small fish-shaped, actively motile organisms. he parasite possesses a long, free, whip-like flagellum at its anterior extremity, and to this, in addition to the possession of an undulating membrane, its power of movement is due.

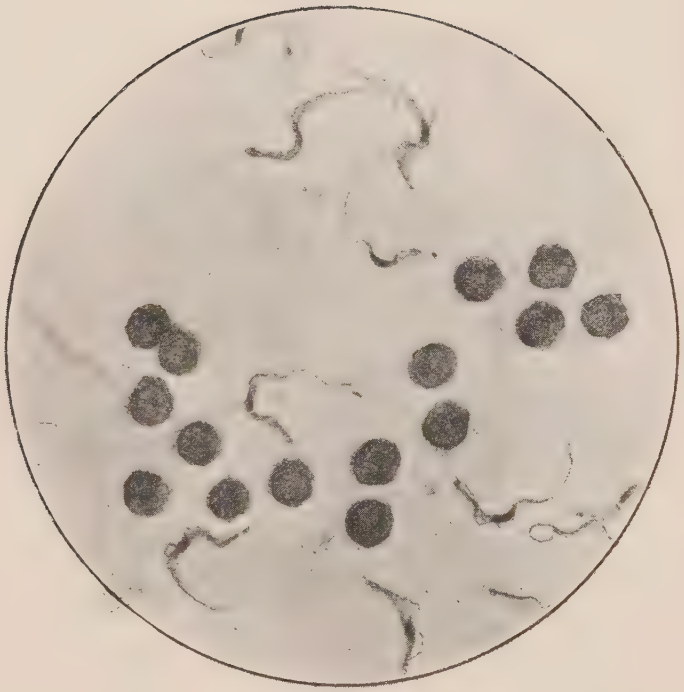


FIG. 30.—MICROPHOTOGRAPH—TRYPANOSOMA BRUCEI IN BLOOD OF RABBIT EXPERIMENTALLY INFECTED (×800).

The first pathogenic trypanosome of animals was discovered in 1880, in the blood of horses affected with surra in India, the discoverer being Evans, an army veterinary surgeon.

Since Evans's notable discovery trypanosomes have been described by other observers as affecting the lower animals in many parts of the world; in fact, the diseases produced by trypanosomes (known collectively as trypanosomiases) are now quite numerous. The most important pathogenic trypanosomes occurring in the domesticated animals are included in the following list:

1. **Trypanosoma Evansi**, the causal agent of the disease surra, affecting horses, mules, asses, camels, elephants, dogs, etc., in India, China, Africa, Mauritius, the Philippines, and other tropical and subtropical countries.

2. **Trypanosoma Brucei**, found by Bruce in the blood of animals suffering from nagana (tsetse-fly disease)—*i.e.*, horses, asses, mules, cattle, dogs, etc.—in tropical Africa.

3. **Trypanosoma equiperdum**, discovered by Rouget in the blood of horses and asses affected with dourine.

This is the only disease due to a trypanosome which so far has appeared on the European Continent.

It occurs in the United States of America and in Africa and India.

4. **Trypanosoma equinum**, the cause of mal de caderas, a South American disease of horses and asses.

5. **Trypanosoma dimorphum**, found by Dutton and Todd in the blood of horses in the Gambia (Gambian horse disease).

6. **Trypanosoma Theileri**, discovered by Theiler in cattle suffering from 'gall sickness' in the Transvaal.

7. **Trypanosoma Transvaaliense**, also described by Theiler as associated with the *Tr. Theileri* in cattle.

Species of Animals attacked by Trypanosomes.

The nagana parasite (*Tr. Brucei*) appears to be most virulent; practically all the lower mammals may be experimentally infected. Horses are extremely susceptible.

Surra especially affects horses and mules, and may be transmitted to most of the lower domesticated mammals. Elephants suffer from the malady, as do also camels, but in the last mentioned it runs a slower course.

Mal de caderas naturally affects horses and asses; the other domesticated animals may be infected experimentally. Cattle are resistant.

Dourine affects particularly horses and asses. Dogs are susceptible to experimental inoculation, but cattle are resistant.

Theiler's trypanosomes (*Tr. Theileri* and *Tr. Transvaaliense*) are peculiar to cattle, and have not, so far, been observed in other animals.

The natural mode of transmission of trypanosomes from one animal to another is by means of biting or blood-sucking insects — in nagana the *Glossina morsitans* (the tsetse fly); in mal de caderas the Stomoxyses; the same in Gambian horse disease; various species of Tabanus in surra; and in gall sickness of the Transvaal the *Hippobosca Rupifes.*

In one disease, namely, dourine, the parasite (*Tr. equiperdum*) is transmitted from one animal to another during coitus—*i.e.*, by the contact of excoriated or ulcerated mucous membranes.

Apparently the above-mentioned trypanosomiases of the lower animals are not transmissible to man. So far only one trypanosome has been discovered in the human being—the *Tr. Gambiense* in West Africa, in cases of 'sleeping sickness.'

Methods of conferring Immunity against the Trypanosomata.

Animals which recover from one attack of trypanosomiasis are, as a rule, immune against a second infection with the same kind of trypanosome.

This is especially true of nagana, and it is against this disease that attempts have been made artificially to confer immunity, but no really successful method has yet been devised.

The serum from nagana-affected animals possessed no immunising properties, and the bile proved equally worthless.

In the case of surra Koch and Schilling endeavoured to attenuate the virulence of the *Trypanosoma Evansi* by passing it through different species of animals, and claimed to have had good results, but Panisset (*Revue Générale de Médecine Vétérinaire*, December, 1904) considers that the facts mentioned by Koch and Schilling are not conclusive. Says Panisset: 'Other observers have found that although no doubt the trypanosomes do become attenuated in their passage through the bodies of certain animals, they recover their original virulency when again inoculated into one of the same species from which they were originally obtained.'

So that at present there is known to us no method of artificially rendering animals immune against infection with the trypanosomata.

Spirochætes.

These are small, extremely thin, and actively motile organisms. The first pathogenic spirochæte was discovered in the blood of human relapsing-fever patients by Obermeier in 1873.

In 1891 Sakharoff, in the Caucasus, discovered the first

pathogenic spirochæte of the lower animals; this has been named the **Spirochæta anserina,** and occurs in the blood of geese, giving rise to a fatal septicæmia. Ducks are also susceptible to infection, but pigeons and sparrows are immune. The disease is experimentally transmissible to geese and ducks by injecting them with blood containing spirochætes. The natural mode of transmission is unknown.

In 1902 Theiler first noticed a spirochæte in the blood of cattle in the Transvaal, and definitely described the organism in 1904. This spirochæte was found in the blood of cattle affected with ordinary red water (due to *P. bigeminum*), and is transmitted by a tick—the *Rhipicephalus decoloratus.* The parasite is not experimentally inoculable either to cattle or other animals.

Spirochætes have also been described in the blood of sheep in the Transvaal by Theiler, and by Martoglio and Carpano in Eritrea.[1]

In 1903 Marchoux and Salimbeni, in Rio de Janiero, discovered in the blood of fowls affected with a fatal septicæmia a spirochæte—The **Spirochæta gallinarum.** Naturally, this parasite is conveyed from fowl to fowl by the *Argas miniatus.* Experimentally it can be transmitted to geese, pigeons, and rabbits, by inoculating them with spirochæte-infected blood.

Birds after recovery from one attack of the disease are immune against a second. According to Blanchard, **immunity** may be conferred on susceptible subjects by passing the blood from a sick fowl through a Berkefeld filter, and inoculating them with the serum so obtained.

[1] On pages 181 to 200 will be found a description of the spirochætes, occurring in cases of 'grease' and 'canker' in horses.

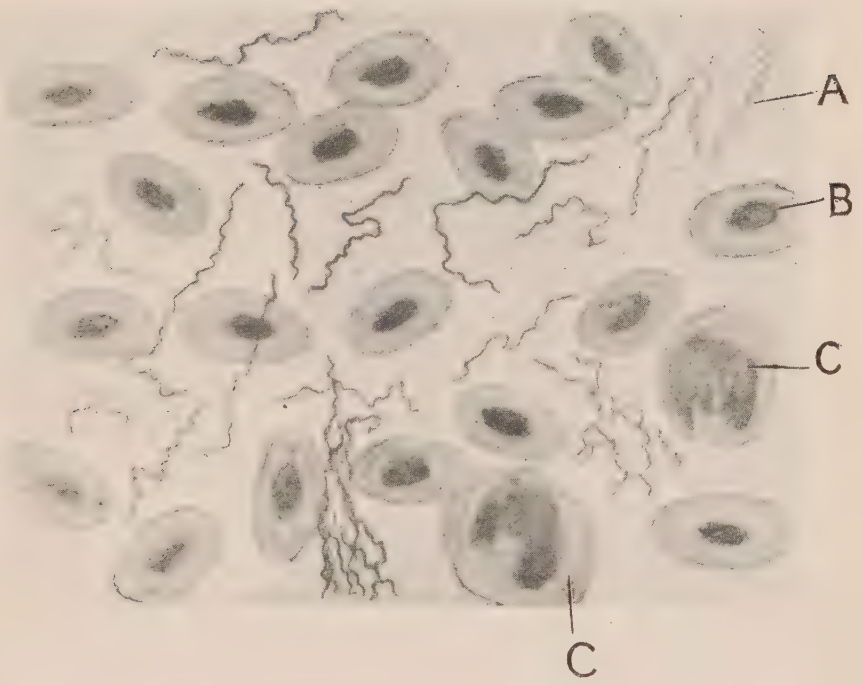


FIG. 31.—SPIROCHÆTE GALLINARUM.

A, Spirochæte ; B, erythrocyte ; C, leucocyte.

Drawn from blood-smear stained by Giemsa's method. Composite field. $\frac{1}{16}$-in. oil-immersion objective. Drawing eyepiece.

CHAPTER V

TOXIN AND SERUM DIAGNOSIS

Tuberculin—Mallein—Serum diagnosis—The agglutination test—The precipitating sera in the examination of meat.

Tuberculin.

Koch's 'old' tuberculin is a sterilised glycerine extract of cultures of the tubercle bacilli containing the products of growth of that organism.

In veterinary practice this agent is used solely as a **means of diagnosing the presence of tuberculosis** in animals. When injected subcutaneously, it is without effect on healthy animals, whilst in tuberculous subjects it produces a marked thermal reaction.

Method of Preparation.

(1) After obtaining pure cultures of tubercle bacilli (and it is immaterial whether we make use of avian or mammalian ; an equally serviceable tuberculin is furnished by either), a quantity of veal bouillon to which has been added glycerine 5 per cent. and peptone 1 per cent. is placed in special shallow flasks (Fig. 32) to insure a free supply of oxygen, and carefully inoculated by placing a small quantity of the original culture *on the surface* of the fluid medium.

(2) The flasks are now placed in an incubator, maintained at a temperature of 38° to 39° C., and left there for from six to eight weeks, until a thick surface growth forms, the bouillon below the growth remaining quite clear.

(3) The flasks are next placed in an autoclave and subjected to steam under pressure at a temperature of 115° C. for twenty minutes to kill the bacilli; afterwards

(4) The volume of the fluid (culture) is reduced to one-tenth its original bulk by evaporation on a steam evaporator.

(5) Finally, the dead organisms are removed by filtration through unglazed porcelain.

The product is known as crude tuberculin; this contains 40 to 50 per cent. of glycerine, and will keep indefinitely.

To test its activity Koch recommended the use of tuberculous guinea-pigs. A good specimen of tuberculin in the dose of 1 centigramme kills a guinea-pig inoculated with tubercle bacilli eight to ten weeks previously; it requires 20 to 30 centigrammes—sometimes even as much as 50 centigrammes—to kill a guinea-pig inoculated only four to five weeks previously—*i.e.*, one in which the disease is less advanced.

The guinea-pigs die in six to thirty hours, according to the extent of the tubercular process.

The preparation known as diluted tuberculin (*tuberculine diluée* of the Pasteur Institute) is generally used for the purpose of testing cattle; this is made by adding 90 parts of a 0·5 per cent. carbolic solution to 10 parts of crude tuberculin.

Dose of Tuberculine Diluée:

For large bulls or bullocks ...	4 c.c.
,, ,, cows...	3·5 ,,
,, medium-sized cows and bullocks	3 ,,
,, heifers and young bulls one to two years old	2 ,,

FIG. 32.—MASSIVE CULTURE OF THE TUBERCLE BACILLUS UPON THE SURFACE OF GLYCERINE BOUILLON, USED IN THE MANUFACTURE OF TUBERCULIN (WITH PERMISSION FROM 'A TEXT-BOOK UPON THE PATHOGENIC BACTERIA,' BY PROFESSOR J. MCFARLAND).

For calves under one year old	...	1 c.c.
,, sheep and goats ...	...	0·5 to 1 ,,
,, pigs	...	1 to 3 ,,
,, ,, under four months old	...	1 ,,
,, ,, from four to nine months old		1·5 to 2 ,,
,, ,, ,, nine to sixteen ,,		2·5 ,,
,, ,, above eighteen months	...	3 ,,

For tuberculin preparations other than the above, those diluted to a greater or less extent, the dose advised by the maker must, of course, be given.

Koch's refined or 'purified' tuberculin is prepared by adding absolute alcohol to the concentrated (crude) tuberculin. A white precipitate forms, and this is collected, washed in alcohol, and then dried. It is of a proteid nature, insoluble in alcohol, but soluble in glycerine and in water. For guinea-pigs it proves fatal in the dose of from 2 to 10 milligrammes. As an agent for the diagnosis of tuberculosis in cattle the dose is 0·4 to 0·5 c.c. Before being used for this purpose it is dissolved in a definite quantity of a 0·5 per cent. solution of carbolic acid.

General Mode of Procedure in testing Animals with Tuberculin.

Experiments by Nocard (1900) prove that a certain time —always more than a fortnight—elapses between the moment of entry of the contagion into the animal system and that at which its effects become manifest by producing a reaction to tuberculin.

McFadyean found that an animal infected with a large dose of bacilli reacted to tuberculin eight days afterwards.

In infection naturally contracted it is doubtful whether animals would react in so short a time.

We see, then, that in animals very recently infected with

tuberculosis no reaction to tuberculin may be obtained; further, it has been proved that very far advanced cases may also fail to react; fortunately, however, in these latter cases the disease may often be diagnosed clinically without the aid of tuberculin.

In testing animals with tuberculin, the first essential is to ascertain the animal's temperature before injection and its usual daily variations.

Once this has been noted, reading the temperature of the reaction offers little difficulty, provided that we 'pay less attention to the height the mercury rises and every attention to the **manner** in which it rises.'

The rise should be **gradual**, the temperature being taken at the ninth, twelfth, fifteenth, and eighteenth hours after inoculation; the maximum point is usually reached from the twelfth to the fifteenth hour, after which it *gradually* falls to normal.

When the thermometric curve is an irregular rise and fall—a sharp sudden rise and sudden fall—such a temperature is not a tuberculous reaction.

Nocard considered a rise of 1·4° F. as insignificant; one of from 1·4° F. to 2·5° F. as suspicious and requiring retesting at the end of a month; while a rise of 2·5° F. to 5·4° F. is characteristic of tuberculosis.

Indications for retesting the animal after the lapse of a month or more are where the temperature curve is suspicious, but not typical, and where a temperature of less than 104° F. is obtained.

The tuberculin is injected subcutaneously, generally in the region of the shoulder or neck.

In animals showing a temperature before inoculation of 103° F. or more the test is not reliable, and should be delayed until the temperature has returned to normal.

Whilst under the test cattle should be kept in a shed, and

fed and watered as usual. Care must be taken that large quantities of cold water are not given to the animal at least during each hour prior to taking the temperature.

Nocard showed that tuberculin has no injurious action on lactation or gestation; nevertheless, animals within three weeks of parturition should not be tested, as misleading variations in temperature may be observed in these.

In old emaciated cows, and in animals tested for the second or third time, double doses of tuberculin should be used.

It is generally considered that by repeated injections of tuberculin a tolerance to this agent is acquired, and for this reason a month or more should be allowed to elapse before retesting.

But **Vallée** has shown that—

1. In the great majority of cases cattle do *not* become accustomed to tuberculin.

2. Tuberculous cattle almost always react to a second injection of tuberculin of *double* the usual dose given, even after an interval of only thirty-six hours after the first dose; but the secondary reaction *commences earlier*, *lasts but a very short time*, and *finishes earlier* than in the ordinary test.

He says (*Revue Générale de Médecine Vétérinaire*): 'The above facts suggest a method of preventing the frauds which have become so common.

'If the veterinary surgeon suspects that a particular animal has been given a dose of tuberculin with the object of defeating the tuberculin test, he should proceed as follows:

'1. About five or six o'clock in the morning inject *double* the dose of tuberculin usually given—of *tuberculine diluée* 8 c.c. for large and 4 c.c. for small-sized cattle may be employed without fear of accident.

'2. *Take the temperatures every two hours from the time of injection* up to the fourteenth or fifteenth hour thereafter.

'3. The intensity of the reaction is indicated by the difference between the temperature at the moment of injection and the highest temperature shown during the following hours.

'4. Any animal giving a reaction of 1·5° C. (2·7° F.) should be considered affected.

'5. A reaction of between 0·8° C. (1·4° F.) and 1·5° C. (2·7° F.) should arouse suspicion.

'6. Animals with an initial temperature of 39° C. (102·2° F.) must not be tested.

'7. Whilst undergoing the test, animals should not be allowed to drink during the hour immediately prior to taking the temperature.'

The Eighth International Veterinary Congress (Buda-Pesth, 1905) passed the following resolutions regarding the **uniform principles for estimating the reaction to the tuberculin test** (when carried out in the usual manner and employing only ordinary, not double, doses):

1. The preparation and supply of tuberculin should be controlled by the State.

2. No animal whose temperature exceeds 39·5° C. (103° F.) is a fit subject for the tuberculin test.

3. A rise of temperature to above 40° C. (104° F.) in any animal whose temperature at the moment of injection was below 39·5° C. is to be regarded as a positive reaction.

4. Any rise in temperature between 39·5° C. (103° F.) and 40° C. (104° F.) must be regarded as of doubtful significance; animals exhibiting such require special study.

Mallein.

Mallein consists of the filtered products of growth of the glanders bacillus. When injected subcutaneously into animals affected with glanders, it gives rise unfailingly to a local and a general reaction, and this is made use of in **diagnosing** the presence of the disease.

Whether mallein has any *curative* properties is a doubtful point; by repeated injections of mallein a tolerance is established, and finally no reaction may be obtained from an animal which had previously given an undoubted glanders reaction. This failure to react, according to some authorities, signifies that the case is cured; according to others, it means simply that a *tolerance* to mallein has been acquired by the animal, although the disease may still be active in the body.

Method of Preparation.

(1) After obtaining a virulent pure culture of the *B. mallei* on agar, this is emulsified with bouillon, and used to inoculate glycerine peptone veal bouillon in wide, shallow, flat-bottomed flasks, similar to the one shown in Fig. 32.

(2) The flasks are placed in an incubator, maintained at a temperature of 37° C. to 38° C., and left there for three or four weeks. By this time the whole surface of the medium will be covered with a yellow-grey growth, and a slight deposit may also have formed at the bottom of the vessel.

(3) The flasks are next placed in an autoclave and submitted to steam under pressure, at a temperature of 115° C., for twenty or thirty minutes, to kill the bacilli; afterwards—

(4) They are removed to an evaporator, and the volume of the fluid (culture) is reduced to one-tenth its original bulk.

(5) Finally it is filtered through porcelain to remove the dead bacilli.

This concentrated mallein, if stored in a cool place, will keep for months ; but before being used for the diagnosis of glanders a definite proportion of a 0·5 per cent. carbolic solution is added to it.

The diluted mallein — *malléine diluée* of the Pasteur Institute—is prepared by adding 90 parts of a 0·5 per cent. solution of carbolic acid to 10 parts of the concentrated mallein.

The dose of *malléine diluée* for horses is 2·5 c.c.

In England a more concentrated preparation is generally used, the dose for horses being 16 minims (about 1 c.c.) to 18 minims or more, depending upon the degree of concentration.

The exact dose as indicated by the manufacturer of the particular brand of mallein used must, of course, always be administered.

In testing mules **double doses** of mallein should be used—the same in retesting *doubtful* cases in horses.

MALLEIN (DRY FORM).—By adding alcohol to concentrated mallein there forms a white precipitate, and this after collection and subsequent washing and drying may be used for the diagnosis of glanders. When required for use, it is dissolved in a definite quantity of a 0·5 per cent. solution of carbolic acid.

General Mode of Procedure in testing Animals with Mallein.

First it is necessary to ascertain the animal's temperature before inoculation, and its usual daily variations.

The mallein is injected subcutaneously in the neck region ; nine hours after inoculation the temperature is taken, and again at the twelfth, fifteenth, and eighteenth

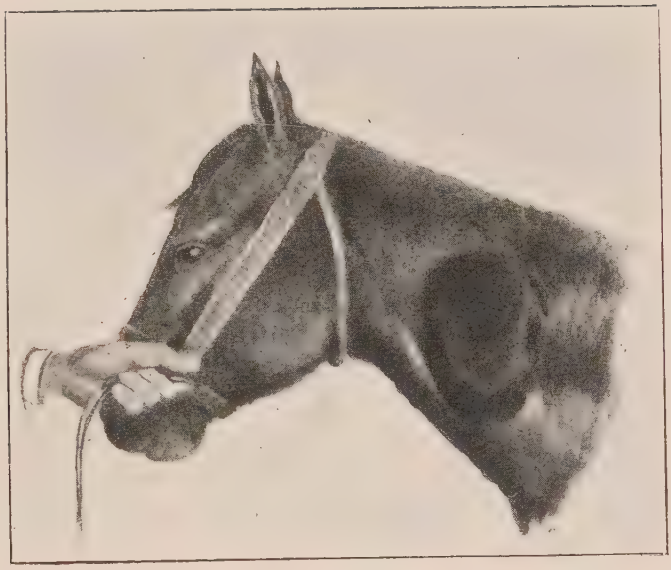


Fig. 33.—Local Reaction to Mallein in a Glandered Subject.

hours thereafter. Cases of delayed reaction occasionally occur in which the thermal rise appears at the eighteenth, twentieth, or twenty-fourth hour, so it is wise to continue taking the temperature for this length of time.

In a glandered animal the temperature will rise 2·6° F. or more during the twenty-four hours following inoculation.

Local Reaction.—In glandered animals a local swelling appears at the site of inoculation within twenty-four hours, *increases in size* for thirty-six hours, and *persists* until the third or fourth day after the injection of mallein. An undoubted swelling is *large*, measuring in diameter from 5 to 10 inches, with thickened, raised edges, and is *painful*.

The injection of mallein into a healthy horse has no effect; a *small* local swelling may arise at the site of inoculation even in non-glandered animals; but this swelling is *not painful*, and instead of continuing to increase after the first twenty-four hours it *diminishes rapidly*, and disappears in thirty-six hours as a rule.

'When the temperature gradually rises from the normal to 104° F. during the first fifteen hours after inoculation, and a large, slowly disappearing swelling forms at the seat of injection, the horse may confidently be declared glandered' (McFadyean).

In doubtful reactions it is advisable to wait three or four weeks before retesting.

Whilst under the test the same precautions should be observed as in testing cattle with tuberculin; the animals should be housed in the stable, receive their usual food, and should not be allowed large draughts of cold water prior to taking the temperature.

Animals showing an initial temperature of 102° F. or more should not be tested; in such cases it is advisable to wait

until the temperature is again normal before performing the inoculation.

The Eighth International Veterinary Congress (Buda-Pesth, 1905) passed the following resolutions regarding the reaction to mallein :

1. Unless the results following the injection of mallein exhibit the characteristics of a typical reaction they must not be regarded as indicating the existence of glanders.

2. A typical reaction comprises a rise in temperature of at least 2° C. The rise would extend above 40° C. (104 F.). During the course of the first day the temperature curve usually exhibits a plateau or two peaks, and on the second, and sometimes even on the third, a more or less marked rise.

This rise in temperature is accompanied by a local and general reaction.

3. Any rise in temperature which falls short of 40° C. (104° F.), and higher atypical reactions, necessitates a second test.

4. A gradual rise of temperature sustained for some time is indicative of glanders, even though it differs from the ordinary type of diagnostic reaction.

5. The local typical infiltration at the point of injection is a certain indication of glanders, even when rise in temperature and the general organic reaction fail.

6. Animals which have undergone the mallein test, whether or not without reaction, should always be tested a second time after an interval of ten to twenty days.

7. The preparation of mallein should only be entrusted to scientific Government institutions, or to institutions recognised and controlled by the State.

8. With the object of determining the full value of mallein, and of clearing the many still unexplained points in regard to the mallein reaction, the Congress requests the

various European Governments to appoint committees to study the question.

SERUM DIAGNOSIS.

The Agglutination Test.

'The serum of animals affected with certain infective diseases possesses the property of agglutinating the microbes which have produced the infection. It is this specific property which furnishes all the elements of serum diagnosis, and this method constitutes a means of diagnosing the existence of a disease otherwise unrecognised—such as latent or 'occult' glanders—or which escapes other means of diagnosis' (Panisset).

The agglutinative phenomenon has been already mentioned (p. 33). The exact nature of the reaction is even yet but imperfectly understood. It would seem to have no direct connection with the subject of immunity, for it makes its appearance early in an infection, before immunity is established.

According to McFarland, 'the agglutinating substance is present in all the normal and pathologic fluids of the infected animal, making its appearance some time after the inception of the process, though occasionally very promptly. It is present throughout the course of the disease, and may remain present for many years afterwards.'

For practical purposes Widal's reaction may be obtained in either of two ways:

1. By adding diluted serum in measured quantity to a known quantity of a freshly made culture of the bacilli, and observing a hanging drop of the mixture under the microscope.

2. By adding the diluted serum in bulk to a suspension of the bacteria in a test-tube, in which case the liquid

becomes gradually clear, the bacilli settling to the bottom as a sediment; this is known as **sedimentation.**

The fluid used to dilute the serum may be either sterile distilled water, normal saline solution, or bouillon.

In every case control tests must be carried out at the same time, using the serum of a **healthy** animal of the same species diluted in the same proportion and with the same diluent.

In the control tubes the solution must remain evenly turbid during the period of observation, and in a hanging-drop preparation the bacilli must be regularly distributed and not collected in clumps (see Fig. 17).

Normal blood-serum shows *some* agglutinative power when added to cultures of many micro-organisms, but with normal sera, as a rule, agglutination only occurs when the dilution employed is slight. If, for example, normal human serum agglutinated cultures of the typhoid bacillus when diluted 1 in 10, the serum of a man affected with typhoid fever might have the same effect when diluted to 1 in 1,000 or more.

Glanders.

In **glanders** McFadyean has shown that when a dilution of glandered blood in salt solution, or broth of 1 in 9, is added to an equal quantity of broth containing the microbes the bacilli form into clumps after an hour. Clumping is complete all over the preparation at the end of two hours.

Rabieux also noted this agglutinative phenomenon in the case of glanders. His method of applying the test is as follows:

1. After collecting the serum, it is diluted with sterile distilled water in proportions varying from 1 in 500 to 1 in 1,500.

2. The **diluted** blood-serum is mixed in small sterile

glass tubes with an equal volume of a culture of glanders bacilli in peptonised bouillon (without glycerine), the age of the culture being from twenty-four to seventy-two hours.

3. The mixture obtained in this way is placed in the incubator at a temperature of 35° to 37° C., and the tubes are examined at variable intervals under the microscope. At the temperature of the room the phenomenon of agglutination appears less rapidly.

He states that when the dilution exceeds 1 in 300 or 1 in 400, non-glandered serum never agglutinates, whereas if the serum came from a glandered animal, agglutination is obtained with dilutions of from 1 in 500 to 1 in 1,000, or even 1 in 1,500.

He considers that whenever a serum exhibits agglutinating properties in the dilution of 1 in 1,000 the animal from which the serum was obtained ought to be considered glandered.

As a rule, with a dilution of 1 in 1,000 the phenomenon does not become distinct until between the eighteenth and thirty-sixth hours. Very exceptionally it required forty-eight hours, but the interval never exceeded seventy-two hours.

When the serum was furnished by a glandered subject **with an elevated temperature** it was found that with a dilution of 1 in 1,500 in two to six hours agglutination manifested itself in very intense fashion. *Rabieux considers this fact of practical importance, since the mallein test cannot be carried out on such a subject.*

Afanasieff found that normal equine serum agglutinised glanders cultures in dilutions of 1 in 400, whilst the serum of horses affected with glanders acted when diluted 1 in 1,600. Wladimirow, Jensen, Bourges and Mery, and Nocard obtained similar results.

Schrürer conducted a number of experiments on 300

healthy and 15 diseased (glandered) horses, the condition of all being verified by post-mortem examination.

Blood was collected from the angular vein of the eye in the two external branches of a small **W**-shaped glass tube. After coagulation the tube was reversed, and the serum collected in the middle branches.

The emulsion used to test the power of the different sera was made from an eight to fourteen days old potato culture of the *B. mallei.*

Before use the emulsion was sterilised at a temperature of 60° C. for three to four hours, and afterwards, for the purpose of obtaining an emulsion identical in every case, lactoscope was used.

The tests were made with measured quantities of the glanderous emulsion to which diluted serum was added.

The mixture, placed in an apparatus similar to that for counting corpuscles, was kept at first for an hour at 52° C. to 54° C., then for twelve to twenty-four to thirty-six hours at the temperature of the incubator.

Schrürer considers that a positive reaction is indicated if agglutination occurs with a dilution of 1 to 2,000. If the result is negative, a second test is made in six to eight weeks.

Kleine and Schmidt, of the Berlin Veterinary College, recently carried out a series of experiments to test the value of agglutination in glanders.

The result of their experiments is reported by Schütz and Miessner in the *Revue Générale de Médecine Vétérinaire*, November 15, 1905.

The method adopted consisted in adding a small quantity of blood from the suspected glandered animal to a suspension of culture of the *B. mallei* in broth. The 'power' or 'degree' of agglutination was estimated by the quantity of blood necessary to produce sedimentation of the bacilli.

Tests were made with blood from 1,911 healthy and 298 glandered horses. The results are shown in the following tables:

Non-Glandered Horses.

1,239 horses (= 64·8 per cent.) had an agglutinative power of 100 to 300.

363 horses (= 19 per cent.) had an agglutinative power of 400.

135 horses (= 7·1 per cent.) had an agglutinative power of 500.

123 horses (= 6·4 per cent.) had an agglutinative power of 600.

41 horses (= 2·2 per cent.) had an agglutinative power of 800.

10 horses (= 0·5 per cent.) had an agglutinative power of 1,000.

0 horses (= 0 per cent.) had an agglutinative power of more than 1,000.

Glandered Horses.

0 horses (= 0 per cent.) had an agglutinative power of 100 to 300.

6 horses (= 2 per cent.) had an agglutinative power of 400.

12 horses (= 4 per cent.) had an agglutinative power of 500.

44 horses (= 14·8 per cent.) had an agglutinative power of 600.

47 horses (= 15·8 per cent.) had an agglutinative power of 800.

75 horses (= 25·2 per cent.) had an agglutinative power of 1,000.

49 horses (= 16·4 per cent.) had an agglutinative power of 1,500.

65 horses (= 21·8 per cent.) had an agglutinative power of 2,000 or more.

It follows from these figures that a horse whose blood agglutinates only in dilutions of 1 per 300, or in dilutions still more concentrated, is free from glanders; whilst horses whose blood agglutinates in dilutions of more than 1 per 1,000 are glandered.

Animals whose blood agglutinates between 1 per 300 and 1 per 500 are **probably** healthy; nevertheless, 6 per cent. of diseased horses in these experiments did not appear to have a higher power.

Animals whose blood agglutinates at 1 per 800 are **probably** diseased, but 2·7 per cent. of healthy ones furnished blood of the same agglutinative value.

Schütz and Miessner formulate the following rules (amongst others) for carrying out the agglutination test:

1. The blood is collected into a sterilised test-tube or flask of 30 to 50 grammes capacity, from the jugular vein, by puncturing it with a trocar, after disinfecting the skin at the site of operation.

2. Animals whose blood cause agglutination at 1 per 1,000 are destroyed.

3. Those giving a reaction at dilutions between 1 to 500 and 1 to 800 are destroyed if they show any suspicious clinical symptoms.

4. If such animals show no clinical symptoms, they are isolated, and if, after a second test, the agglutinative power is increased, they are destroyed.

5. But if they show no reaction at the second test they are considered non-glandered.

Leclainche, in reviewing the subject of the serum diag-

nosis of glanders, affirms that the test is incomparably inferior to mallein. He states: 'Taking into account all possible errors which may have been ascribed to the mallein test, yet these are twenty times less than those attending agglutination.'

Now, mallein certainly is a most valuable diagnostic agent, yet, under certain circumstances, one may desire confirmatory evidence; in such cases serum diagnosis might be indicated, but, above all, *it can be used when for any reason the mallein test is inapplicable*, as, for example, in subjects with an elevated temperature. Again, according to Bonome, the injection of mallein into a glandered subject markedly increases the agglutinative power of its blood-serum; this is noticed during the continuance of the mallein reaction, but *it occurs even in animals which, through repeated injections, fail to give a definite reaction to mallein*, and in these cases the test now under consideration would furnish a valuable aid to diagnosis.

Finally, in the examination of dead subjects where the glanders lesions have been fradulently removed prior to the inspection, the agglutinative test is further indicated.

In view of the technical character it is likely to remain a laboratory method of diagnosis.

Tuberculosis.

The serum diagnosis of **tuberculosis,** attempted by a number of workers, has not proved successful.

In the first place, it is not easy to obtain a uniform suspension of tubercle bacilli—they have a natural tendency to collect together in clumps—and even when this obstacle has been surmounted, the agglutination test in the disease now under consideration is not specific, the reaction occurring often in the same dilution, whether healthy or affected (tubercular) blood-serum is used.

Arloing and Courmont employed in the test eight to twelve days old cultures of tubercle bacilli in glycerine bouillon, and added 1 part of fresh blood-serum from a suspected animal to 10 parts of culture; clumping became apparent in from two to twenty-four hours. Their experiments were conducted on fifty healthy and seventy tuberculous animals, post-mortem examinations being held in every case after testing.

They declared that blood-serum from healthy animals had no agglutinative power on emulsions of tubercle bacilli when diluted in the proportion of 1 in 10, whereas serum obtained from diseased (tuberculous) animals furnished a positive reaction when dilutions of *at least* 1 in 10 were used.

Nocard, Leclainche, Beck, Rabinowitsch, Panisset, and others, have proved conclusively that the agglutination test in the case of tuberculosis is not specific, and serum diagnosis in this disease is of no value in veterinary practice.

Swine Fever.

Dawson first applied Widal's reaction to the diagnosis of swine fever.

In this case a fresh twenty-four hours old culture of the *B. choleræ suis* is required, and from this is made a uniform suspension of the microbes in bouillon. A platinum loopful of the latter is then placed on a slide and ten times the quantity of diluted serum (1 in 10) from the suspected animal added.

A hanging-drop preparation of this mixture is next examined through the microscope; it is stated that in less than thirty minutes, if the case be one of swine fever, the bacilli lose their motility and collect in clumps.

The agglutinative test for swine fever is of doubtful

value ; the *B. coli communis* and some other organisms respond to it quite as readily as the swine fever bacillus.

In certain other diseases of animals—*strangles*, *swine plague*, *fowl cholera*, etc.—the agglutinative reaction has been applied as a method of diagnosis by Jess, Piorkowsky and Ostertag, but their results have been somewhat uncertain, and lack confirmation by other workers.

The Precipitating Sera in the Inspection of Meat.

The precipitating sera have been utilised in the examination of meat with a view to detecting adulteration of minced and smoked flesh — sausages, mince - meat, etc., the adulterants in such cases being usually either horse, cat, or dog flesh, or the meat of fœtuses.

Von Rigler conducted a series of experiments to test the value of this method. Rabbits were injected every three days for a period of one month with 5 to 10 c.c. of watery extracts of the meat of seven species of animals (horse, ox, pig, cat, hare, rabbit, and roebuck), each rabbit receiving only an extract from one species of animal.

He found that whilst normal rabbit serum produced no precipitate when added to aqueous extracts of meat, the antisera obtained from treated rabbits gave rise to a definite reaction.

This reaction consists in the formation of a precipitum when the antiserum is added to a filtered watery extract of the same kind of flesh used in inoculating the animal, whilst no precipitation is obtainable with the muscle extract of a different species of animal.

A mixture of several extracts does not interfere with the result, provided always that the necessary variety of muscle extract is included in the mixture.

The reaction is not absolutely specific, as it may occur with *related* species of animals; thus the serum of a rabbit treated with horse flesh may produce a precipitate with horse and donkey flesh, but this hardly limits the value of the test in the practice of meat inspection.

Notel, in investigating the same subject, obtained satisfactory results, as have also certain other authorities, notably Vallée and Nicholas.

The animal which is to furnish the antiserum should receive periodic injections of the particular kind of meat extract against which it is desired to obtain a testing serum, and at frequent intervals during the process samples of blood should be withdrawn, and the precipitating activity of the serum tested on homologous meat macerations.

When a sufficient degree of activity has been attained, the animal should be allowed to rest for a few days, then killed, and its serum collected, under aseptic precautions, into previously sterilised glass tubes. Obtained in this manner, and subsequently stored in a cool, dark place, it will retain its activity for a considerable length of time.

'Whether for the purpose of inoculation or simply for testing, it has been established that prolonged aqueous macerations are preferable to maceration in saline solution.'

According to Nuttall, an extract is suitable for testing when it foams on being shaken.

In performing the test, after extracting the flesh with distilled water and filtering, it is necessary to add to the filtrate an equal volume of salt solution exactly double the strength of normal saline (see Appendix A, 'Normal Saline Solution'). A small quantity of the fluid is now placed in a clean glass tube and the testing serum added.

The reaction effected at ordinary room temperatures consists in the immediate production of a well-marked cloudiness. Later flocculi collect, sink, and form a distinct

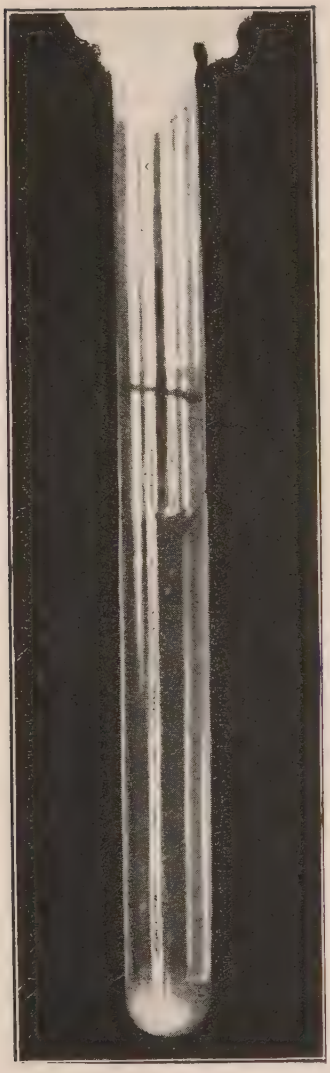


FIG. 34.—PHOTOGRAPH OF TUBE CONTAINING AN AQUEOUS EXTRACT OF HORSE FLESH AND DOUBLE NORMAL SALINE SOLUTION, EQUAL PARTS.

(Horse flesh is present in proportion of 1 to 19 of liquid.)

To this was added serum from a rabbit which had received eleven injections of horse flesh extract at intervals of three days.

A well-marked precipitum is shown.

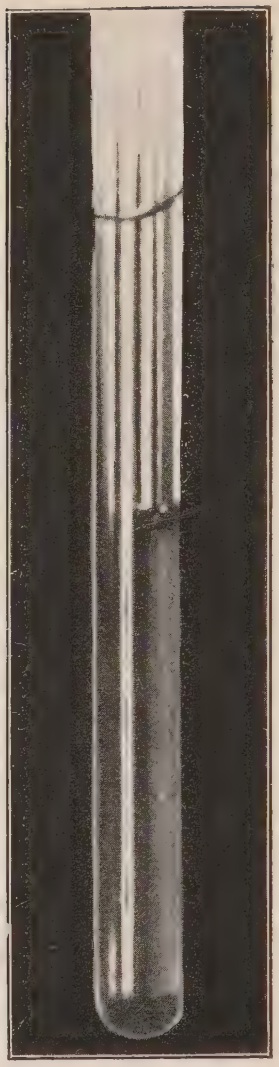


FIG. 35.—PHOTOGRAPH OF TUBE CONTAINING EQUAL PARTS OF SALINE SOLUTION (DOUBLE NORMAL) AND AN AQUEOUS EXTRACT OF PORK —'SAUSAGE-MEAT.'

(Pork present in proportion of 1 to 19.)

To this was added serum from a rabbit previously injected with horse flesh extract (the same rabbit used in previous experiment, Fig. 34). In this case the solution has remained quite clear.

No precipitum.

greyish precipitum at the bottom of the test-tube (see Fig. 34).

Martel (Paris) affirms that 'the considerable activity of the musculo-precipitants leads to the admission of a **unit** (in estimating the reaction) capable of answering to the following conditions: The serum from animals injected with 10 per cent. macerations of healthy bruised flesh should be active in the quantity of 0·25 c.c. (about 5 drops) when added to 10 c.c. of an experimental maceration of 5 per cent. (1 part flesh to 19 parts of distilled water), the macerations for injection or experiment being completed in five hours at ordinary temperatures.'

The reaction of sero-precipitation furnishes the meat inspector with a most precise and delicate method of identifying the flesh of animals, and in time, no doubt, the test will be more generally used to detect the fraudulent adulteration of the minced meats, sausages, etc., so extensively sold for human consumption.

APPENDIX A

1. Weights and Measures.

1 cubic centimetre (1 c.c.)	= 16·23 minims.
28·35 c.c.	= 1 fluid ounce.
100 c.c.	= approximately $3\frac{1}{2}$ ounces.
568 c.c.	= 1 pint.
1 litre (1,000 c.c.)	= 1·76 pints (35 ounces).
1 metre	= 39·37 inches.
1 centimetre	= approximately $\frac{2}{5}$ inch.
2·54 centimetres	= 1 inch.
1 millimetre	= approximately $\frac{1}{25}$ inch.
1 gramme	= 15·432 grains.
28·4 grammes	= 1 ounce avoir.
1 kilogramme	= 2·205 pounds avoir. (approximately 2 pounds 3 ounces avoir.).

2. Thermometric Scales.

On the centigrade (C.) scale the freezing-point of water is made zero and the boiling-point 100, whilst on the Fahrenheit (F.) scale the freezing-point is placed at 32° and the boiling-point at 212°.

A difference of 1° C. is equal to that of 1·8° F.

FORMULA FOR THE CONVERSION OF THE DEGREES OF ONE SCALE INTO THOSE OF ANOTHER.

If above the freezing-point of water (32° F., 0° C.)—

F. into C. = (Degree − 32) ÷ 9 × 5.
C. into F. = Degree ÷ 5 × 9 + 32.

3. Normal Saline Solution (Physiological Salt Solution).

This is prepared by dissolving 0·75 per cent. of sodium chloride in distilled water.

APPENDIX B

NOTIFIABLE DISEASES

IN Great Britain and Ireland the following diseases are scheduled under the Contagious Diseases (Animals) Acts :

Rinderpest (cattle plague).
Pleuro-pneumonia (bovine).
Sheep-pox.
Foot and mouth disease.
Swine fever.
Glanders.
Anthrax.
Rabies.
Epizootic lymphangitis.
Sheep scab.

Any person having, or having had, in his possession or under his charge an animal affected with or suspected of any of the above-mentioned diseases must notify the local authorities with all practicable speed. Equine scabies is

also scheduled in Ireland and in some districts in Great Britain, and in addition there is an order—the Dairies, Cowsheds, and Milkshops Order of 1899—bearing on tubercular mammitis in cows.

In the case of rinderpest, pleuro-pneumonia, foot and mouth disease, sheep-pox, and swine fever, affected and 'in-contact' animals are slaughtered and compensation paid to the owners. The regulations prohibit movement of animals in infected places for a specified length of time after the outbreak, and order thorough disinfection of the premises, utensils, etc., and burial or destruction of diseased carcasses.

In rinderpest and pleuro-pneumonia slaughter of all affected and 'in-contact' animals is *compulsory*.

All sheep affected with sheep-pox must be destroyed, but sheep suspected only of being affected, or of having been exposed to infection, *may* be destroyed by the local authorities '*if they think fit*.'

In swine fever the Board of Agriculture **may**, 'if they think fit,' slaughter affected or in-contact animals.

The regulations relating to foot and mouth disease are also permissive—*i.e.*, the Board may, 'if they think fit,' destroy both affected and in-contact animals, and pay compensation to the owners.

As a matter of fact, in all these diseases (rinderpest, pleuro-pneumonia, sheep-pox, swine fever, and foot and mouth disease) on the occurrence of an outbreak all affected and 'in-contact' animals are invariably slaughtered. With the exception of swine fever, these diseases are at present non-existent in the United Kingdom; they have been 'stamped out.' And now, taking into account our insular position, fresh outbreaks can be more economically and satisfactorily dealt with by a continuance of the same measures.

The following remarks by Colonel Smith ('Veterinary

Hygiene') are eminently applicable to the subject now under consideration:

'The beneficial effects of inoculation as a means of stamping out the disease in a country like the United Kingdom cannot for a moment be entertained. Communications are too extensive, the country too small, and sources of leakage too numerous, to trust to any system but that of ruthless obliteration. In our other possessions, especially those occupied by an alien race, the destruction of diseased animals is not understood, and is resented; it has even been known to provoke a native rising, and in such cases our obvious policy should be something less effectual, but also less drastic, and fortunately the vast areas and the slow communications are in this respect of help. Under these circumstances inoculation must be practised.'

The above remarks are made in respect of pleuro-pneumonia, but they apply equally to rinderpest, sheep-pox, foot and mouth disease, and swine fever in the United Kingdom.

The **glanders** order prohibits the movement of diseased animals, and enforces disinfection of premises in which the disease has existed.

It *permits* local authorities, 'if they think fit,' and the owner is willing, to slaughter diseased or suspected animals.

The local authorities cannot compel slaughter, but they can shut up an owner's premises so long as a diseased animal remains therein.

The regulations recognise as diseased only those animals showing the characteristic external symptoms of glanders, and as suspected only those showing external lesions of a doubtful nature.

The veterinary inspector has no power to test animals with mallein. If the owner consents to this course, the test may be applied, but not otherwise.

The **anthrax** order provides for inspection by local authorities, and prohibits movement into or out of any place wherein an animal has died from anthrax, until such place has been thoroughly disinfected, and no animals remaining therein are affected with the disease.

It prescribes disinfection of premises, provides for burial or destruction by cremation or chemical agents of diseased carcasses, and prohibits movement of diseased or suspected animals.

In **rabies** the local authorities can compel the destruction of a rabid dog, or one having been bitten by such an animal, and the isolation of all dogs exposed to infection. Regulations may be prescribed for the cleansing and disinfection of any place used for the diseased or suspected dogs, and of anything used for or about these animals.

The order can be extended to apply to ruminants, equines, and swine, and if diseased or suspected, the authorities may order their destruction and pay compensation.

The Importation of Dogs Order enacts that no dog may be introduced into the United Kingdom without a license from the Board of Agriculture. When landed, the dog must be detained, and, at the expense of the owner, isolated for six months on the premises of a veterinary surgeon.

Tuberculosis.— The only regulation bearing on this disease is the Dairies, Cowsheds, and Milk Shops Order of 1899, which provides that—

'If at any time "disease" exists among the cattle in a dairy or cowshed, or other building or place, the milk of a diseased cow therein

'(*a*) Shall not be mixed with other milk;

'(*b*) Shall not be sold or used for human food; and

'(*c*) Shall not be sold or used for food of swine or other animals, unless and until it has been boiled.

'In the case of (*a*) and (*b*) these shall apply to diseases of the udder certified by a veterinary surgeon to be tubercular.'

The word 'disease' in this order means only the scheduled contagious diseases of animals.

The veterinary inspector has no power to test animals with tuberculin, unless and until he has obtained the owner's consent so to do.

BIBLIOGRAPHY AND REFERENCES

Bosanquet: Immunity and 'Serums, Vaccines, and Toxines.'

Bowhill: Bacteriological Technique and Special Bacteriology.

Bulloch: Immunity. Journal of Comparative Pathology and Therapeutics.

Cadiot: Clinical Veterinary Medicine.

Friedberger and Frohner: Veterinary Pathology.

Hektoen and Riesman: Text-book of Pathology.

Hewlett: Serum Therapy; Bacterial Therapeutics and Vaccines.

Hunting: Journal of Comparative Pathology and Therapeutics, and Veterinary Record.

Jess: Kompendum der Bakteriologie und Blut-Serum Therapie.

Law: Veterinary Medicine.

Legge: Milroy Lecture delivered before the Royal College of Physicians, London, 1905: Industrial Anthrax.

McFadyean: Journal of Comparative Pathology and Therapeutics.

McFarland: A Text-book upon the Pathogenic Bacteria.

Metschnikoff: The Harben Lectures for 1906.

Nuttall: Blood Immunity.

Smith, F.: A Manual of Veterinary Hygiene.

Stockman: Preventive Inoculation and the Serum Methods.

Theiler: Report to the Transvaal Department of Agriculture, 1904; Journal of Comparative Pathology.

Wassermann: Immune Sera. Hæmolysins, Cytotoxins, and Precipitins.

'Annales de l'Institut Pasteur.'

'Bulletin of the United States Bureau of Animal Industry.'

'Journal of Comparative Pathology and Therapeutics.'

'Journal of the Transvaal Department of Agriculture.'

Proceedings of the National Veterinary Association, 1905.

'Recueil de Médecine Vétérinaire.'

Report of the Departmental Committee appointed by the Board of Agriculture to inquire into the Etiology, Pathology, and Morbid Anatomy, and other matters connected with the Diseases of Sheep known as Louping Ill and Braxy.

'Revue Générale de Médecine Vétérinaire.'

'The Agricultural Journal of the Cape of Good Hope.'

'The British Medical Journal.'

'The Journal of Preventive Medicine.'

'The Lancet.'

'The Veterinary Journal.'

'The Veterinary Record.'

'Veterinary News.' Etc.

NOTE ON THE OCCURRENCE OF SPIROCHÆTÆ IN CASES OF 'CANKER' AND 'GREASE'*

THE *Spirochæta Obermeirei*, found in the blood of man in relapsing fever, was discovered by Obermeier in 1873, whilst the *Spirochæta anserina* (the first pathogenic spirillum found in animals) was described by S. Sakharoff. In 1903 Marchoux and Salimbene described a spirillosis of fowls in Rio de Janeiro caused by the *Spirochæta gallinarum*, and in 1904 Theiler described a spirillosis of cattle in the Transvaal. Recently the *Spirochæta pallida* has been found in syphilis, and other spirochætæ in certain other diseased conditions in man.

I now desire to call attention to the occurrence of a spirochæte in horses in cases of so-called 'canker' and 'grease.' I first noticed this organism on November 11 last in examining a case kindly placed at my disposal by Mr. Henry Sumner, M.R.C.V.S., of Liverpool. In preparations made by taking some of the diseased material from either of the cases mentioned, adding a drop of distilled water on a slide and placing a cover-glass over all, on examination with a $\frac{1}{12}$-in. oil-immersion objective the micro-organisms are easily detected by their active undulating movements.

After staining, the organism is found to have a corkscrew

* Reprinted from the *Veterinary Record*, December 2, 1905.

or spiral shape, and varies considerably in length and breadth. *Length*, 7 to 20 μ or more; *breadth*, about $\frac{1}{4}$ μ or even less. Each microbe shows the same thickness throughout, except at its extremities, where it thins somewhat. *Number of spirals* 5 to 10 (occasionally a greater number is observed, but the majority show a number included in these figures). Occasionally a less number of spirals is seen, and in some cases none whatever, the organism appearing as a straight line.

In a wet film spirals are observed in every case. Possibly during the process of drying the specimen and 'fixing' these may be effaced.

Although the spiral or corkscrew forms predominate in stained specimens, some may appear S-shaped, looped, semicircular, figure-of-eight, etc., depending on the position finally obtained during drying. Tangles are common.

The number of organisms present in each case varies considerably. I have, however, noticed that they bear a distinct relation to the gravity of the case—in well-marked cases very plentiful, whilst in others present in fewer numbers.

In all well-marked cases of 'canker' and 'grease' so far examined no difficulty has been found in discovering their presence.

Staining.

Giemsa's stain gives good results, the organisms appearing as *red* coloured lines and spirals.

Schaudinn's modification is an excellent method, but requires eighteen to twenty-four hours' staining. In this case they appear bright red.

Romanowsky's method, after eighteen hours, stains beautifully, again a red colour. No flagellum, centrosome, or nucleus could be detected.

Methylene blue (1 per cent. solution) and carbol fuchsin

also stain, but the results in these cases are not very satisfactory.

The organism does not stain by the Gram or Claudius methods. In all stained preparations variations are noticed in the staining reactions, the thinner (younger?) forms staining less intensely than those better developed.

Löffler's flagella stain shows in some cases an undulating membrane, and flagella were also observed on a number of the spirilla. By this method of staining another phenomenon is observed—some of the microbes appear to be in the process of longitudinal division, whilst occasionally two almost completely developed were found lying side by side attached only at one of the extremities.

Some of the organisms also showed a beaded appearance. I can offer no opinion as to the nature of this last reaction.

Cultivation.

Cultivation on the usual media was quite unsuccessful. This has also been found the case in the above-mentioned spirochætes of man and animals.

The material obtained by scraping the diseased tissues was collected in sterile test-tubes containing a small quantity of water at the bottom to prevent drying. By this means the organisms preserved their activity for days, and I believe they also multiplied.

Inoculation.

Inoculation into rabbits, guinea-pigs, rats, pigeons, etc., gave negative results.

In veterinary literature it is freely admitted that 'grease' can be inoculated in the horse from one leg to the other, and that 'canker' may originate from 'grease' and *vice versâ*.

Whether the spirochætes now described are the actual cause of 'canker' and 'grease' is a debatable point. Until

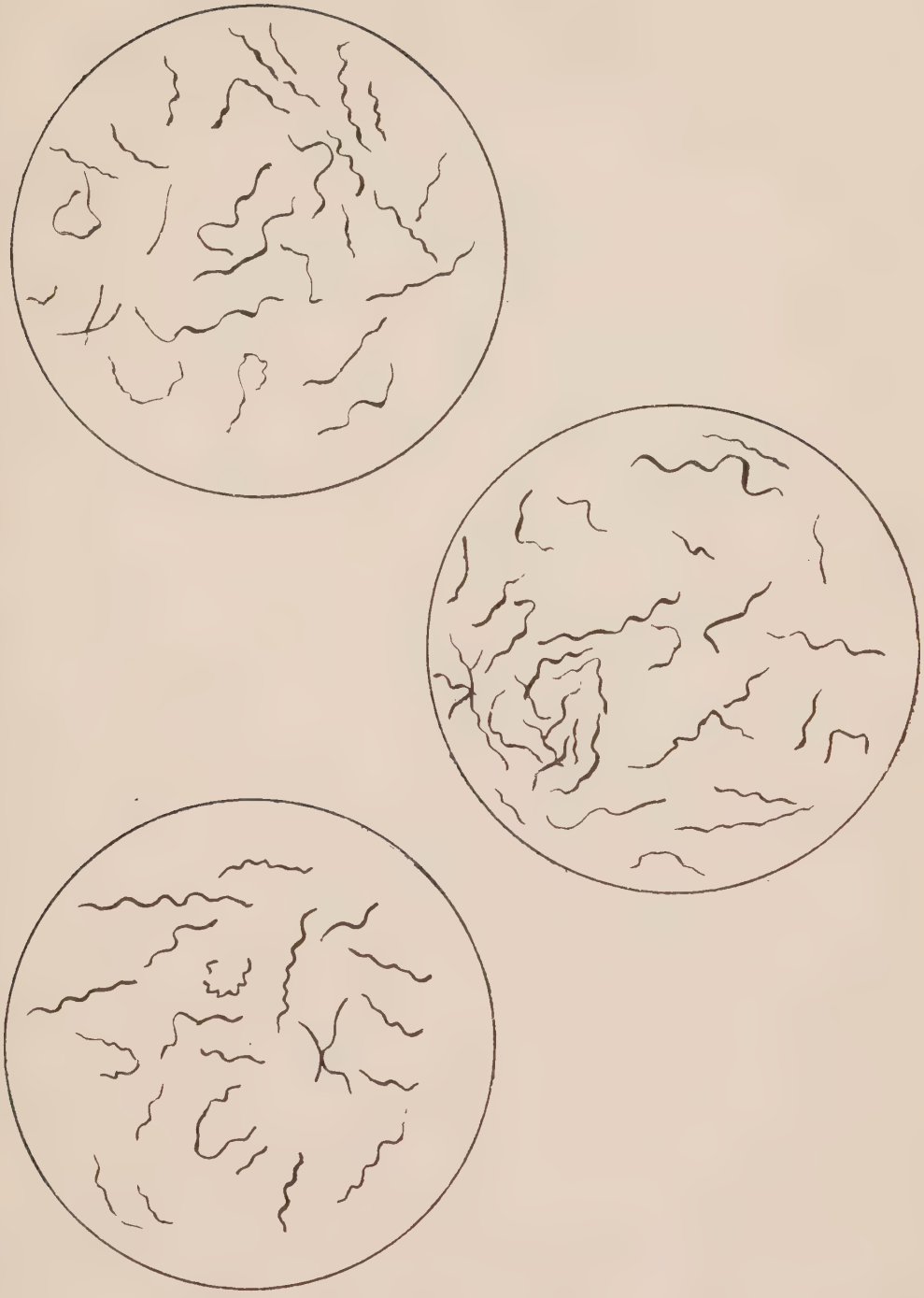


FIG. 36.—SMEAR FROM A CASE OF 'CANKER' (THREE FIELDS).

Drawn from specimen under microscope. Leitz $\frac{1}{12}$-in. oil-immersion objective. Ocular No. 4.

FIG. 38.—SHOWING THICK SPIRILLA STAINED WITH GENTIAN VIOLET; ALSO A FEW THIN SPIROCHÆTES.

Leitz $\frac{1}{16}$-in. oil-immersion objective. Ocular No. 4. Composite field. Slightly enlarged.

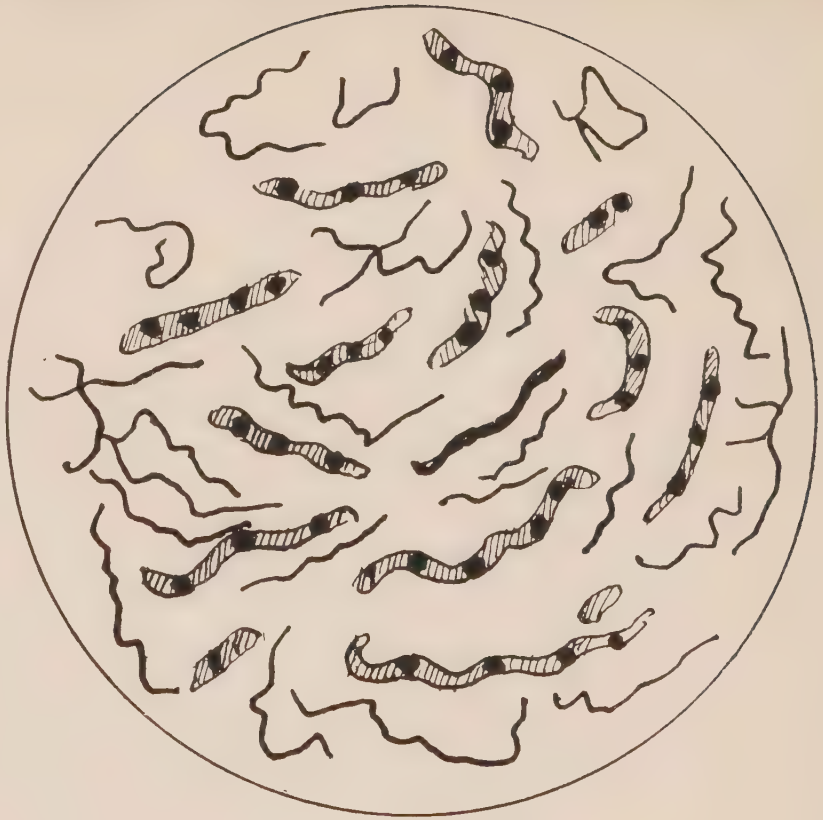


FIG. 39.—SHOWING THICK SPIRILLA; ALSO A FEW THIN SPIROCHÆTES.

Giemsa and Romanowsky's stains. Leitz $\frac{1}{16}$-in. oil-immersion objective. Ocular No. 4. Composite field. Slightly enlarged.

FIG. 40.—SHOWING THICK SPIRILLA WITH FLAGELLA.

Löffler's stain. Leitz $\frac{1}{16}$-in. oil-immersion objective. Ocular No. 4. Composite field. Slightly enlarged.

FIG. 41.—THICK SPIRILLA.
(Division forms ?)

Löffler's stain. Leitz $\frac{1}{16}$-in. oil-immersion objective. Ocular No. 4. Composite field. Slightly enlarged.

their cultivation on artificial media and isolation have been successfully accomplished, and subsequent inoculation into healthy horses has reproduced the disease, it is impossible to affirm with certainty that they are the actual causal agents.

So far I have noted that—

1. The spirochætæ now described are constantly associated with the diseases 'canker' and 'grease.'

2. They were not observed in smears from any other equine diseased material.

3. The number of organisms present bears a distinct proportion to the gravity of the case.

ADDITIONAL NOTE ON THE OCCURRENCE OF SPIRILLA IN EQUINE 'CANKER' AND 'GREASE.'*

In the microscopical examination of smears from the diseased tissue in these cases three forms of spiral organisms may be distinguished:

1. The spirochæte described in the *Veterinary Record*, December 2, 1905.

2. A spirochæte differing from the above in its reaction to Giemsa's stain, appearing as faint *blue* thin lines and spirals.

3. A much thicker, 'stumpy' spirillum; this organism, when stained with Giemsa and Romanowsky's stains, shows a blue body, and in some of the microbes darker circular areas (usually three or four) can be distinguished (Fig. 39).

It is considerably thicker than the other forms; generally only two or three spirals are seen, although elongated forms occur having a greater number, and in other straight forms

* Reprinted from the *Veterinary Record*, December 9, 1905.

the spirals are apparently almost effaced in the process of drying.

In contrast to the other two forms, this organism stains well with methylene blue (1 per cent. aqueous solution) and carbol fuchsin, and is not entirely decolorized by the Gram method of staining. By means of Löffler's stain terminal flagella can be demonstrated (Fig. 40).

Correction.— In my previous article the paragraph referring to the demonstration of flagella by means of Löffler's stain should refer only to the *thick* spirilla described in the present note, and not to the *thin* spirochætæ.

A FURTHER NOTE ON THE OCCURRENCE OF SPIROCHÆTÆ IN CASES OF EQUINE 'CANKER' AND 'GREASE.'

In the *Veterinary Record* of December 2 and 9, 1905, I called attention to the presence of spirochætæ in the diseased material obtained from cases of 'canker' and 'grease.'

I then forwarded specimens to Berlin, to the well-known authority Dr. Schaudinn, with a request that he would inform me whether, in his opinion, these spirochætæ resembled any of the organisms found in man. He states in his reply :

'Your forms [of spirochætæ] are different from the following species : (1) *Spirochæta pallida*, (2) *S. refringens*, (3) *S. buccalis* (Dentium), (4) *S. angina vincate*, (5) *S. Obermeieri.*'

He considers that they have more likeness to the spirochætæ found in ulcerous processes in man, especially in ulcerous carcinomata.

FIG. 42.

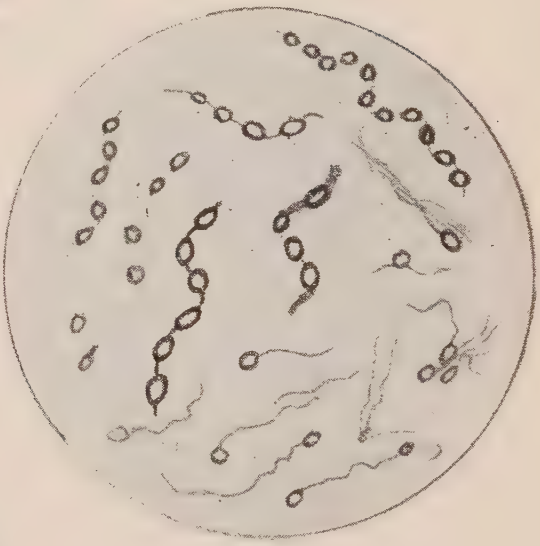


FIG. 43.

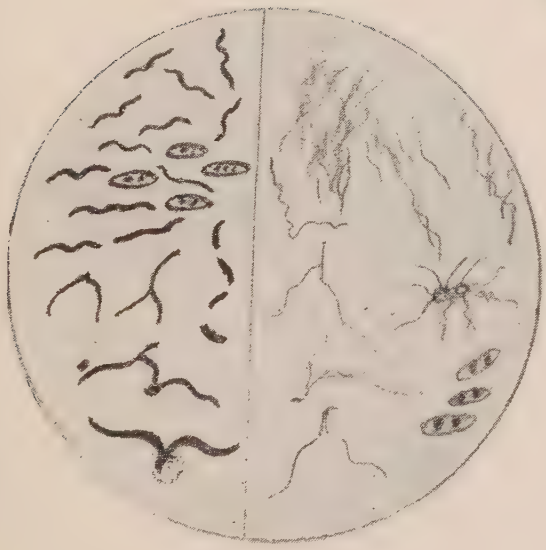


FIG. 44.

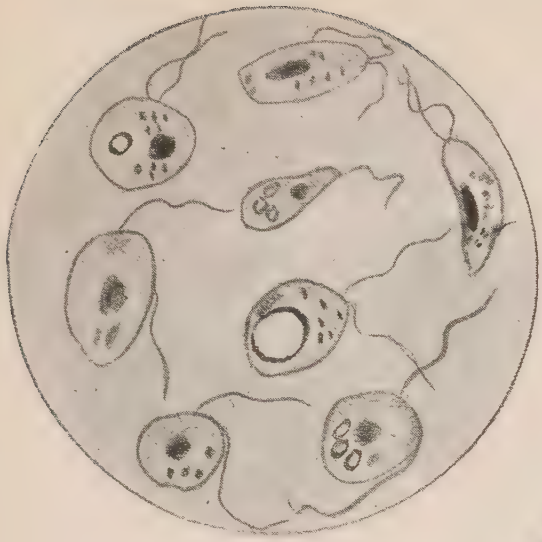


FIG. 45.

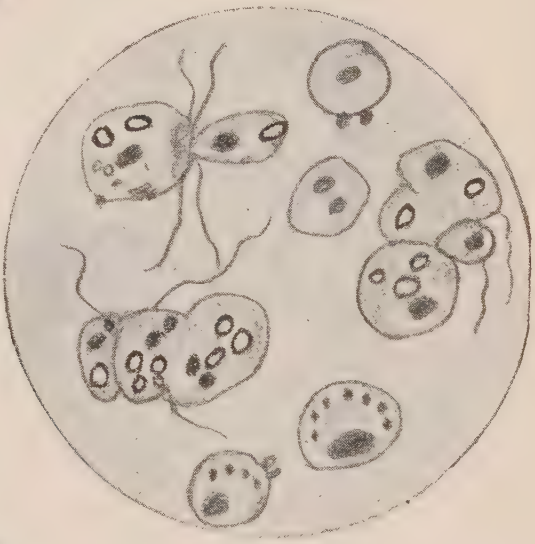
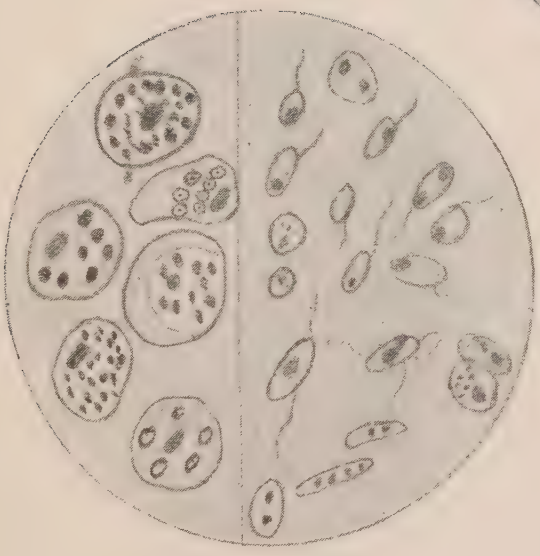


FIG. 46.



A. B.

FIG. 47.

'They resemble in their variability the species—or, as I believe, the several species—which are found in ulcerous processes of the skin, such as skin carcinomata.

'But,' he adds, 'our knowledge of the spirochætæ is still too small to allow of a systematic classification. In the meantime the question of their identity with one of the numerous species occurring in man must be left an open matter.'

I may say that, since I first noticed the spirochætæ, I have continued to examine all cases of equine canker and grease with which I have come in contact, and have never failed to find the organism present in more or less abundance.

Further, I have examined material from many other equine diseased conditions—such as ulcerous processes of the skin, 'quittors,' sinuous wounds of the withers (fistulous withers), 'poll evil,' etc.—but in no cases except those of 'canker' and 'grease' have I yet observed spirochætæ. During the past months I have attempted the cultivation of these organisms, and now desire to illustrate the various forms as seen on cultivation.

In 'canker' and 'grease' I think we may roughly group the spiral organisms there present into two chief forms (*vide Veterinary Record*, December 9, 1905)—(1) the long thin spirochætæ, (2) the thicker 'stumpy' spirilla.

The drawings (Figs. 42, 43, and 44) are from specimens, both stained and unstained, prepared from various cultures and examined microscopically; they include some of the various forms so far observed.

In addition to the spirochætæ already described, I have also constantly noted the presence of flagellate organisms apparently of the orders Monadida and Heteromastigida (*Protozoa*, class *Mastigophora*; subclass *Flagellidida*). These are oval or round, of varying appearance and staining

reaction. The majority possess two flagellæ, some, however, only show one, whilst others (developmental forms?) appear to have lost their flagellæ. Smaller organisms (Fig. 47 B) are also seen.

In the drawings (Figs. 45, 46, and 47) I have endeavoured to show specimens of some of the various forms as seen under a $\frac{1}{16}$-inch oil-immersion lens, either unstained or after staining by Romanowsky's method.

ADDITIONAL NOTE.

For the cultivation of the organisms described in the foregoing notes a semi-liquid medium of alkaline reaction has been employed. This was prepared by mixing with blood-serum from the horse a quantity of nutrient agar.

On this medium *mixed* cultures have been maintained during the past months, but so far all attempts to *isolate* the spirochætes have proved unsuccessful.

W. J.

November, 1906.

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Baillière, Tindall and Cox, 8, Henrietta Street, Covent Garden